Marta Luísa Gonçalves de Freitas Pereira

O funcionamento executivo como indicador de conversão para a doença de Alzheimer: contribuição da análise do movimento ocular

Tese apresentada à Faculdade de Medicina da Universidade de São Paulo para obtenção do título de Doutor em Ciências

Programa de Psiquiatria

Orientador: Prof. Dr. Orestes Vicente Forlenza

São Paulo
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À minha “Luisinha”
(*in memoriam*)

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List of Abbreviations

AD	Alzheimer's disease
ADRDA	Alzheimer's Disease and Related Disorders Association
AIC	Akaike criterion
aMCI	Amnestic MCI
ANOVA	Analyses of variance
AS	Anti-saccade
Aβ1–42	Amyloid-β1–42
BADS	Behavioural Assessment of the Dysexecutive Syndrome
BDS	Backward Digit Span
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
COWAT	Controlled Oral Word Association Test
CSF	Cerebrospinal fluid
CTRL	Control
DLPFC	Dorsolateral prefrontal cortex
DSM-V	Diagnostic and Statistical Manual of Mental Disorders – V
EF	Executive functions
EOG	Electro-oculography
FAPESP	Fundação de Amparo à Pesquisa do Estado de São Paulo
FAS	Verbal Fluency test
FDG	Fluorodeoxyglucose
FDS	Forward digit span
FEF	Frontal eye fields
I-KF	Kalman Filter
IGT	Iowa Gambling Test
IPq-HCFMUSP	Instituto de Psiquiatria / Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo
LIM-27	Laboratório de Neurociências
LIP	Lateral intraparietal area
MCI	Mild cognitive impairment
mdMCI	Multiple domain MCI
MoCA	Montreal Cognitive Assessment

naMCI	Non-amnestic MCI
NCD	Neurocognitive disorder
NINCDS	National Institute of Neurological and Communicative Disorders and Stroke
p-tau	Phosphorylated tau 181
PEF	Parietal eye field
PET	Positron emission tomography
PPC	Posterior parietal cortex
PS	Prosaccade
RAVLT	Rey Auditory Verbal Learning Test
ROC	Receiver Operating Characteristics
ROI	Regions of interest
SC	Superior colliculus
SCi	Intermediate layers of the superior colliculus
SCs	Superficial layers of the superior colliculus
SD	Standard deviation
sdMCI	Single domain MCI
SEF	Supplementary eye fields
SNpr	Substantia nigra pars reticulata
SPSS	Statistical Package for Social Sciences
Stroop C	Stroop ‘Colour’
Stroop W	Stroop ‘Word’
Stroop WC	Stroop ‘Word-Colour’
t-tau	Total tau
TMT	Trail Making Test
VOG	Video-oculography
WAIS-R	Wechsler Adult Intelligence Scale - Reviewed
WCST	Wisconsin Card Sorting Test

Resumo

Pereira MLGF. *O funcionamento executivo como indicador de conversão para a doença de Alzheimer*: contribuição da análise do movimento ocular [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2019.

INTRODUÇÃO: O objetivo do estudo foi caracterizar o desempenho no funcionamento executivo em sujeitos controles saudáveis, sujeitos com comprometimento cognitivo leve e sujeitos com a Doença de Alzheimer (AD), através da análise do movimento ocular. Ainda que diversas métricas do movimento ocular tenham sido relacionadas com o declínio cognitivo na demência, pouco se sabe sobre a sua associação com o funcionamento executivo prévio à AD, nomeadamente no envelhecimento normal e no comprometimento cognitivo leve (MCI). MÉTODOS: 93 sujeitos idosos completaram avaliações clínica e neuropsicológica e foram alocados em três grupos, de acordo com o seu *status* cognitivo: controles (CTRL, n=28), com comprometimento cognitivo leve (MCI, n=44) e com Doença de Alzheimer (AD, n=21). Todos os grupos foram testados com um protocolo de movimento ocular, composto por uma tarefa de pró-sacada (PS) e uma de anti-sacada (AS). A tarefa de PS requeria uma sacada rápida e automática em direção a um estímulo alvo periférico. Na tarefa de AS, os sujeitos tinham que inibir uma PS automática em direção ao alvo e iniciar uma sacada voluntária na direção oposta ao alvo (hemicampo oposto). A tarefa de AS é conhecida por ser uma medida precisa do funcionamento executivo. Ambas as tarefas tinham três sub-condições: simples (o ponto de fixação central desaparecia imediatamente antes do surgimento do alvo), gap (o ponto de fixação central era removido 200ms antes do surgimento do alvo) e overlap (o ponto de fixação desaparecia um pouco depois do surgimento do alvo). RESULTADOS: A análise do movimento ocular na tarefa de PS revelou um padrão de desempenho sacádico semelhante em todos os grupos. A tarefa de AS teve um impacto maior na frequência e na latência das métricas do movimento ocular em cada grupo, com os sujeitos AD mostrando um declínio executivo maior do que os CTRL e com o grupo MCI desempenhando de forma intermédia. O grupo MCI encontrava-se afetado de forma semelhante ao grupo AD nos tempos de reação das sacadas voluntárias, com um tempo prolongado de correção de sacadas errôneas em direção ao alvo. Correlações e regressões revelaram relações significativas entre as métricas do movimento ocular na AS e medidas cognitivas de controlo inibitório, atenção, velocidade de processamento, memória operacional e auto-monitoramento. A análise das curvas ROC sugeriu que o movimento ocular revela uma boa acurácia na distinção entre os grupos. CONCUSÕES: Os três grupos demonstraram um padrão de movimento ocular semelhante na tarefa de PS, o que revela um controle sacádico automático preservado no envelhecimento normal, no MCI e na AD. A tarefa de AS confirmou que a AD afeta os padrões de movimento ocular, refletindo déficits executivos no controle inibitório, memória operacional e controle executivo da atenção, necessário na execução de sacadas voluntárias. Pacientes com MCI revelaram um prejuízo intermediário nestes domínios das funções executivas. Contudo, eles apresentaram tempos de resposta significativamente aumentados, à semelhança dos sujeitos com AD, por forma a contornar estes déficits.

Descritores: Função executiva; Doença de Alzheimer; Comprometimento cognitivo leve; Movimentos oculares; Sacadas.

Abstract

Pereira MLGF. *Executive functioning as an indicator of conversion to Alzheimer's disease*: the contribution of eye movement analysis [thesis]. São Paulo: "Faculdade de Medicina, Universidade de São Paulo"; 2019.

INTRODUCTION: The objective of this study was to characterise executive functioning performance in healthy control subjects, subjects with mild cognitive impairment and subjects with Alzheimer's disease (AD) through the analysis of the eye movement behaviour. Although eye movement metrics have been related to cognitive decline in dementia, little is known about its association with executive functioning prior to AD, namely in healthy ageing and in mild cognitive impairment (MCI). METHODS: 93 elderly individuals completed clinical and neuropsychological evaluations and were allocated into three groups according to cognitive status: normal controls (CTRL, n=28), mild cognitive impairment (MCI, n=44) and Alzheimer's disease (AD, n=21). All groups were tested with an eye movement protocol composed of a prosaccade (PS) and an anti-saccade (AS) task. The PS task required a fast, automatic saccade towards a peripheral target stimulus. In the AS task, subjects had to inhibit an automatic prosaccade towards the target stimulus and initiate a voluntary saccade towards the opposite direction of the target (opposite hemifield). The AS task is known to be an accurate measure of executive functioning. Both tasks had three sub-conditions: simple (the central fixation point disappeared immediately before the onset of the target), gap (the central fixation point was removed 200ms before the onset of the target) and overlap (the fixation point disappeared shortly after the onset of the target). RESULTS: Eye movement analyses in the PS task revealed a similar pattern of saccadic performance across the three groups. The AS task had a greater impact on the frequency and latency of eye movement metrics in each group, with AD subjects showing a greater executive decline than CTRL and with MCI group performing intermediately. MCI were similarly impaired as AD in their voluntary saccadic reaction times, with a longer time to correct an erroneous saccade towards the target. Correlations and regressions revealed significant relationships between eye movement metrics in the AS and cognitive measures of inhibitory control, attention, processing speed, working memory and self-monitoring. ROC curve analysis suggested that eye movements reveal a good accuracy in distinguishing between the groups. CONCLUSIONS: The three groups showed a similar pattern of eye movements in the PS task, revealing a preserved automatic saccadic control in healthy ageing, MCI and AD conditions. AS task confirmed that AD affects eye movement patterns reflecting executive deficits in inhibitory control, working memory and executive-attention control needed when executing voluntary saccades. MCI patients revealed an intermediary impairment in these executive function domains. However they showed significant increased response times similar to AD subjects, in order to overcome these deficits.

Descriptors: Executive function; Alzheimer's disease; Mild cognitive impairment; Eye movements; Saccades.

1. INTRODUCTION

INTRODUCTION

Alzheimer's disease (AD) has been a hot topic amongst the international scientific community, which has dedicated great efforts to detect the early signs of the disease. The most recent studies indicate the presence of other types of early cognitive alterations in addition to the well-described changes in episodic and semantic memory. There seems to be an early impairment of executive functions, however the extent to which different executive sub-domains are affected is yet to be known (Clark et al., 2012; Schott et al., 2010).

Eye movement measures have proved to be a powerful tool in exploring higher cognitive processes. This is high precision, non-invasive technique that requires minimal verbal or motor involvement from the subjects. Hence the analysis of eye movement has been suggested as a potential accurate surrogate method of assessing cognitive impairments in the course of AD. Previous studies showed promising results in detecting executive dysfunction in AD patients (Garbutt et al., 2008; Crawford et al., 2005; Mosimann et al., 2004). Although encouraging, studies focusing on eye movement behaviour in mild cognitive impairment (MCI) subjects with executive deficits are still almost non-existent.

The present study seeks to clarify which eye movement paradigms and oculomotor metrics best describe subtle changes in the various sub-domains of executive functions in healthy aged controls, MCI and AD participants.

This study aims to bring a relevant contribution to the description of early cognitive decline in pre-symptomatic stages of AD and to contribute to the identification of potential preclinical markers of the disease.

2. LITERATURE REVIEW

2.1. Brief history of eye movement research

Vision, and more specifically eye movement behaviours, have been a topic of research for centuries, with the first descriptions dating back to Ancient Greece. Nonetheless, it was only in the 18th century that more systematic eye movement studies started to be implemented. William Charles Wells (1757-1817) contributed to the description of vertigo with his studies using an afterimage (created by the prolonged exposure of the eyes to a bright light; the subject kept seeing the image even after closing the eyes) to study the dislocation of the eyes (Wade & Tatler, 2011). Jan Evanglista Purkinje (1787-1869) extended Well's experiments on eye movement patterns in visual vertigo using a novel method with electrical stimulation of the ears. It was during this experiments that Purkinje made the first description of nystagmus (Wade, 2007).

Years later, Hermann Helmholtz (1821-1894) gave another invaluable contribution to eye movement research. Using a bite bar for controlling head position, this researcher studied the geometry of eye movements in a more accurate way. Helmholtz defined the planes of the head with regard to the symmetry between the two sides (median plane) and the line joining the two eyes (transverse plane). In addition, he defined primary, secondary and tertiary eye positions to measure ocular rotation (Wade, 2007).

Ewald Hering (1834–1918) was one of the first researchers interested in binocular eye movements. According to Hering, eye movements acted as a single unit, with both eyes moving as an 'imaginary single eye'. He also described fixations occurring between eye movements, drawing attention to how the eyes reach a specific point. In addition, he developed new methods of measuring eye movements by placing rubber tubes on the eyelids to capture the sound produced by contractions of the

ocular muscles. These ‘clapping’ sounds were found during reading and they would disappear when the eyes fixated on a stationary target. With this method, Hering described the class of rotations, nowadays known as saccadic eye movements and he was a pioneer studying the discontinuity of eye movements in other research areas such as reading (Wade & Tatler, 2011).

The first description of ‘saccades’ is attributed to Hering’s coeval Louis-Émile Javal (1839-1909), when he briefly mentions in one of his texts that the eyes make quick movements, called saccades, over each line of a text while reading. Javal had several unsuccessful attempts of registering eye movements and it was only in 1879 that the first saccadic movements were recorded by Lamare during reading. This experiment was only described years after and it had a remarkable similarity to the technique created by Hering in 1879 (Wade, 2007).

It was still during the nineteenth century that the development of eye-tracking techniques emerged. The first eye-trackers contained a lever attached to an eye-cup to register eye movements on the surface of a smoked drum. Apart from the mere visual observation of the eye, these initial techniques involved a direct contact with the cornea and they were invasive to the human eye. However, researchers quickly progressed to devices that photographically recorded eye movements, where a light was reflected directly from the surface of the eye itself (Dodge & Cline, 1901 *apum* Wade, 2007). Dodge’s findings were not only crucial for the study of eye movements and visual perception but they traced a path to the proliferation of eye-movement research that was observed in the following years. Soon eye-tracking technique was extended to research areas other than reading. The saccade-fixation pattern was applied in other tasks associated with eye movement and cognition (Wade & Tatler, 2011).

Decades later Buswell and Yarbus started to investigate the association between eye movement behaviour and high-level cognitive domains. In one of his experiments, Yarbus recorded eye movements while individuals viewed Repin's painting, *The Unexpected Visitor* (1884). In this experiment, participants viewed the painting several times but with a different instruction each time: a) free-viewing, b) capture the material circumstances of the family, c) estimate the ages of the people, d) guess what the family had been doing before the arrival of the unexpected visitor, e) describe the clothes worn by the people in the painting, f) remember the positions of the people and objects and g) estimate how long the visitor had been away from the family. Yarbus' ground-breaking findings showed that eye movement patterns were significantly different in the free viewing condition and under different instructions given to the observer (Wade & Tatler, 2011).

The author concluded that eye movement patterns are influenced not only by visual properties of the elements present in a stimulus but also by the task and its goals (top-down control). Moreover, Yarbus found that the observer's gaze showed a particular preference towards the human figure and particularly towards the eyes more than other features of a face, a finding that had been previously achieved by Buswell. These authors concluded that the eyes fixate on regions of interest of a scene with relevant information for the decision making process and that our gaze is dependent on the cognitive task being performed (Borji & Itti, 2014).

The twentieth century brought an extensive development in eye movement research, with more realistic and complex stimuli being used in experiments. With this progress, a new demand of more advanced eye tracking technologies started to emerge in the second half of the century. From bulky and invasive devices that were confined to laboratories, eye trackers progressed to head-mounted cameras that

recorded a film of the observer's gaze and then finally to mobile and non-invasive, video-based devices (Barreto, 2012). The advent of computational methods started a new era of eye movement data collection and data analysis, creating endless opportunities for eye movement research. Despite all the advances made in the last centuries, innumerous questions are still yet to be answered in eye movement research, namely about the association between eye movements and cognition and how this association reflects brain pathology in many neurodegenerative conditions. In the next chapter, a more detailed description of the different types of eye movements will be presented.

2.2. Types of eye movements

The study of eye movement represents an accurate and reliable "window" to the brain in clinical and research environments. Eye movement patterns during visual scanning are characterised by a sequence of saccades and fixations. Different eye movements can be distinguished by their physiological properties and they can contribute to a better diagnosis of several diseases with oculomotor abnormalities, such as neurodegenerative conditions (Leigh & Zee, 1999). This way, six eye movement behaviours can be described depending on whether they hold images of a stimulus steady on the retina or if they direct the fovea to an object of interest. The first category includes (1) fixations: hold a stationary stimulus on the fovea when the head is not moving; (2) vestibulo-ocular reflex: holds images of the a target steady on the retina during brief head movements; and (3) optokinetic: holds a target stimulus on the retina during sustained head movements. When looking at systems that direct the fovea to an object of interest, it can be described (4) vergence: moves the eyes in an opposite direction (i.e., convergence or divergence) so that images of a single

object are placed simultaneously on both foveas; (5) smooth pursuit: holds a moving stimulus on the fovea and helps gaze stabilization during sustained head movements; and (6) saccades: brings the image of an object of interest onto the fovea (Wong, 2008).

For the purpose of this study, the present review will focus on a brief description of saccadic movements. Saccades are the fastest of eye movements and enable us to redirect our line of sight and redirect the fovea to objects of interest. Saccades are involved in several different oculomotor behaviours from reflexive movements initiated by a novel visual stimulus to high-order planned gaze shifts toward the remembered location of a visual target (Leigh & Zee, 1999).

Saccadic behaviour includes a wide variety of types of saccades described in table 1. Saccades are usually combined with other eye movements such as vergence (eye rotations in opposite directions), as well as head and limb movements.

Table 1. Classification of saccades

Classification	**Description**
Volitional saccades	Elective saccades made as part of purpose behaviour.
- Predictive, anticipatory	Saccades generated in anticipation of or in search of the appearance of a target at a particular location.
- Memory-guided	Saccades generated to a location in which a target has been previously present.
- Anti-saccades	Saccades generated in the opposite direction to the sudden appearance of a target.
- To command	Saccades generated on a cue.
Reflexive saccades	Saccades generated to novel stimuli that unexpectedly occur within the environment.
Express	Very short latency saccades that can be elicited when the novel stimulus is presented after the fixation stimulus has disappeared.
Spontaneous saccades	Seemingly random saccades that occur when the subject is not required to perform any behavioural task.
Quick phases	Quick phases of nystagmus generated during vestibular or optokinetic stimulation or as automatic resetting movements in the presence of spontaneous drift of the eyes.

2.3. Neuroanatomy of the saccadic system

Saccades are considered ballistic movements - with an average duration of 100ms - and there is no time for visual feedback, so accuracy is dependent on internal monitoring of neural signs. There is a proportional relationship between its amplitude, speed and duration. Therefore, any interference in these associations indicates abnormalities in saccadic movements (Leigh & Kennard, 2004). A brief description of the neurophysiology and neuroanatomy controlling saccadic movements can be found in the following sections.

In order to process an image the brain needs to transform the stimulus, which is encoded by active neurons within the visual cortex into the saccadic command on ocular motoneurons, which is encoded in terms of frequency and duration. In addition, a transformation from retinal coordinates into brain coordinates is mandatory.

The generation of saccades depends on six extraocular muscles responsible for the stabilization of the image. The motoneurons located in these extraocular muscles receive signals (intense discharge) for saccades from cells in the brain stem called ‘premotor burst neurons’. This burst or pulse of activity occurs before and during saccade performance and is needed to make the eye move rapidly (Leigh & Kennard, 2004). At the end of the saccade, the motor neurons fire at a slower rate and the contraction of extraocular muscles allows the eye to be hold steady in a new position (Leigh & Zee, 1999). During fixation, omnipause neurons are active and their activity is cancelled during saccade performance (Keller et al., 2000).

Accuracy of saccades greatly relies on the role of the *cerebellum*. The dorsal vermis (lobule VII) and the caudal part of the fastigial nucleus are essential elements in the accuracy of saccades. Specifically, neurons in the fastigial nucleus precisely encode the time when a saccade must slow down when it is close to the target.

Previous findings revealed that the early activity in one fastigial nucleus accelerates the eyes in the opposite direction and that later activity in the other fastigial nucleus stops the eye on the target, facilitating fixation. A possible delay of the later activity will cause hypermetria, because the eye will not decelerate and it will overshoot the target (Robinson et al., 1993).

The above described cerebellar regions related to saccadic accuracy also project to the cerebral cortex via the thalamus. Inactivation or impairments in the dorsal vermis, the fastigial nucleus or in the thalamus impact the adaption of saccades to new visual demands (Leigh & Kennard, 2004).

The superior colliculus (SC) plays an important role in the spatial-temporal control of visual fixation and saccadic eye movements. The superficial layers of the SC (SCs) contain neurons that receive inputs from the retina as well from other visual areas (Robinson & McClurkin, 1989). Neurons in the intermediate layers of the SC (SCi) project to critical structures in the brain stem that generate the premotor commands for saccades (Munoz et al., 2000). Neurons that increase their discharge before and during saccades, referred to as saccade neurons, are distributed throughout the SCi. Neurons that are active during visual fixation and paused during saccades, referred to as fixation neurons, are located in the rostrolateral area of the SC where the fovea is represented. These saccade and fixation neurons in the SC project directly to the brainstem premotor circuitry in the reticular formation to determine behaviour (Glimcher, 2001).

The SCi receives information from different cortical areas, specifically posterior parietal and frontal cortex, and basal ganglia which all play a role in the voluntary selection of potential saccadic targets to determine behaviour. Visual inputs that are

crucial for maintaining fixations or generating saccades are directed from the visual cortex, through the parietal lobe, to the SCi (Glimcher, 2001 for detailed reviews).

Visual information is processed through different extrastriate areas before it arrives in motor areas to perform the action. One area in particular is the lateral intraparietal area (LIP). LIP projects to both the intermediate layers of the SC (SCi) and frontal cortical oculomotor areas including the frontal eye fields (FEF), the supplementary eye fields (SEF), and the dorsolateral prefrontal cortex (DLPFC). The FEF play a crucial role in executing voluntary saccades. The SEF play an important role in internally guided decision-making and sequencing of saccades. The DLPFC is involved in cognitive processes such as executive functions, spatial working memory, and the inhibition of automatic responses (Coe & Munoz, 2017).

In fact, planning saccades depends on different cortical and subcortical areas. These include: the FEF, the SEF, the pre-SEF (located just anterior to the SEF) and the parietal eye field (PEF) which corresponds to the LIP in monkeys. Other important areas for saccade programming are the DLPC (located on the dorsolateral surface of the frontal lobe, anterior to the FEF) and the posterior parietal cortex (PPC, Leigh & Zee, 1999).

Frontal and parietal cortical areas project directly to the SC and frontal areas project indirectly through a basal ganglia pathway that includes the caudate nucleus and *substantia nigra pars reticulata* (SNpr). The frontal areas also project, via pontine nuclei such as *nucleus reticularis tegmenti pontis*, to the dorsal vermis and fastigial nucleus of the *cerebellum* (Hikosaka et al., 2000).

In the next section, it will be briefly discussed how these different cortical areas contribute to different types of saccades, specifically automatic / reflexive saccades and to volitional / anti-saccades.

2.4. Neuroanatomical mechanisms involved in different types of saccades

2.4.1. Automatic / Reflexive saccades

The human eye moves in an effortless way and produces innumerous spontaneous saccades within a fraction of a second. Many of these saccades are externally guided by visual stimuli and a have a very short duration (less than 100ms). Some researchers recreated these express saccades by introducing a temporal gap between fixation offset and the target onset. In result, a short-latency reflexive saccade was produced before the presentation of a new stimulus (Fischer & Ramsperger, 1984). The PEF projects to the SC and plays an important role in initiating reflexive saccades. After PEF lesions in humans (but not after an FEF or an SEF lesion), latency of visually triggered saccades is significantly increased, especially when the lesion involves the right cerebral hemisphere (Pierrot-Deseilligny et al., 1991).

The status of express saccades as a separate type of saccade is far from being consensual in the literature. Express saccades were first addressed as a product of a relatively simple neural circuit involving the rostral pole of the SC and parietal cortex (Reuter-Lorenz et al., 1991). However, other authors argued that the direction (but not latency) of express saccades can be influenced by higher level cognitive processes concerning task instructions (Edelman et al., 2007).

2.4.2. Volitional / anti-saccades

The ability to suppress reflexive responses and to generate voluntary motor commands is crucial for everyday life, specifically in the achievement of internal goals.

The anti-saccade (AS) task has been used extensively to investigate mechanisms of voluntary saccade control. A peripheral visual stimulus appears and the subject must suppress the impulse to look toward the visual stimulus and make a

saccade to the opposite direction of the stimulus (Coe & Munoz, 2017). This task will not be extensively discussed in this section, as it will be further analysed later in this literature review.

Both the FEF and DLPC are involved in programming AS behaviour. Thus, functional imaging studies have shown that FEFs are activated bilaterally during AS. The right hemisphere DLPC is also activated during AS. Deficits in each of these cortical areas have a different impact in AS oculomotor measures. Thus, patients with discrete lesions affecting the DLPC exhibited an increased percentage of errors in the AS test. On the other hand, patients with FEF lesions had a normal percentage of errors on the AS task, but they show an increased latency when performing a correct AS. Therefore, it has been suggested that, during the AS task, inhibition of reflexive, erroneous saccades is due to the DLPC, whereas triggering of the intentional, correct AS is dependent on the FEF (Pierrot-Deseilligny et al., 2003).

In conclusion, saccadic movements are a product of an extensive network of brain areas. It can be added that a better understanding of the neuroanatomical substrates of eye movements, specifically saccades, is crucial to unveiling the cognitive processes behind our actions and intentions. Ultimately, saccadic eye movements are a useful and non-invasive tool that can be used to tease apart deficit in different clinical conditions. Next, a short description of different eye tracking systems will be presented.

2.5. Eye tracking systems

Eye tracking is the most common technique of measuring eye movements. There are three broad categories of gaze estimation methodologies. Gaze estimation can be accomplished by (1) measuring electric potentials using electrodes placed

around the eye (electro-oculography, EOG), (2) measuring movements of a search coil embedded in a scleral contact lens attached to the eye, and (3) observing eye movements from online/offline recorded images of the eye (video-oculography, VOG), (Duchowski, 2007). This brief review of eye tracking systems will mainly focus on VOG, as it was the chosen technique in the present study.

The anatomy of the eye allows light to be captured by the retina and simultaneously be reflected out it. VOG uses these light reflections, also known as the Purkinje reflections (Leigh & Zee, 1999). The corneal reflection of the light source (typically infra-red) is measured relative to the location of the pupil center.

Two points of reference on the eye are needed to separate eye movements from head movements. The positional difference between the pupil center and corneal reflection changes with eye rotation, but remains relatively constant with small head movements. An infra-red light source is usually used, as it is invisible to the human eye, and hence non-distracting. Because the infra-red light source is usually placed at some fixed position relative to the eye, the Purkinje reflections are relatively stable as the pupil rotates in its orbit (Duchowski, 2007).

Video-based systems offer spatial accuracy without the discomfort and disruption to participants observed in other eye tracking systems. In remote eye trackers, the recording device is positioned to a location further from the participant's eye.

By reducing the necessity to fully restrain the head of the participant, the range of applications and participant groups with which eye tracking experiments can be conducted with ease is much increased (Wade & Tatler, 2011). Saccades are easy to measure and quantify, and responsive to computational approaches. With non-invasive video-based eye tracking systems large datasets can be captured in a

reduced amount of time, which makes this technique particularly suitable for clinical populations, such as people with neurodegenerative conditions. Moreover, it allows the design of shorter, less stressful cognitive assessments for patients.

2.6. Alzheimer's disease

Alzheimer's disease (AD) is currently the most common form of dementia in the elderly population accounting for 50% – 75%, roughly doubling in prevalence every 5 years after age 65. Recent reports suggest that nearly 47 million people may have AD worldwide, making it one of the leading cause of death in western countries (Prince et al., 2014; Ziegler-Graham et al., 2008). It is estimated that 74.7 million people will live with dementia by 2030 and 131.5 million by 2050, according to the World Alzheimer Report 2015 (Prince et al., 2015). The same trend has been observed in Brazil, where the prevalence of dementia is 7.1%. AD accounted for 55.1% of the cases, vascular dementia 9.3% and AD with cerebrovascular disease 14.4% of all cases (Herrera, 2002).

The gold standard for the diagnosis of AD is an autopsy-based (post-mortem) pathological evaluation. The neuropathological markers of AD are well defined, namely the presence of senile plaques and neurofibrillary tangles associated with the excessive production and aggregation of the amyloid b peptide and the hyperphosphorylation of Tau protein in affected neurons (Castellani et al., 2006; Nelson et al., 2009). AD patients have specific neuroanatomical features such as atrophy of the temporal cortices, including the amygdala, hippocampus, and inferior temporal lobes, as well as of the anterior cingulate cortex (Poulin et al., 2002).

The Diagnostic and Statistical Manual of Mental Disorders – V (DSM-V - American Psychiatric Association, 2013) has reclassified AD as a 'major

neurocognitive disorder' (NCD), which also considers earlier stages of cognitive impairment as 'mild neurocognitive disorder'. A major NCD has to reflect prejudices in one or more cognitive domains (attention, executive function, learning and memory, language, perceptual-motor function, social cognition) and the deficits must not be due to another mental disorder. In addition, loss of independent functioning must be present in major NCD. According to the DSM-V criteria, Dementia caused by AD can be further classified as: (1) Probable Alzheimer's disease dementia (when the person meets all of the core clinical criteria); (2) Possible Alzheimer's disease dementia (when there is an atypical or mixed presentation or (3) Probable or possible Alzheimer's disease dementia with evidence of the Alzheimer's disease pathological process (when there is biomarker evidence that highlights this association).

In parallel, the National Institute on Aging-Alzheimer's Association has also updated the set of criteria for the clinical diagnosis of AD, established originally by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) in 1984 (McKhann et al., 1984). In this new approach, these workgroups have considered criteria for the diagnosis of pre-clinical and prodromal stages of the disease. All the extensive knowledge developed around the pathophysiological processes of AD in the last 27 years was considered, namely biomarker evidences that may enhance the specificity of the diagnosis of AD. These findings may include reduced levels of amyloid-β1–42 (Aβ1–42); elevations of total tau (t-tau) and phosphorylated tau 181 (p-tau181) in the cerebrospinal fluid (CSF); atrophy in medial, basal and lateral temporal lobe and medial parietal lobe measured by structural magnetic resonance; and a decrease in fluorodeoxyglucose (FDG) uptake on PET in temporo-parietal cortex (McKhann et al., 2011).

AD typically begins in an insidious manner and it is difficult to precisely date the onset of the clinical symptoms. Usually it remains undetected by many families due to the preservation of basic abilities (e.g., language and sensory motor skills) in the early stages of the disease. In this early, pre-clinical stage of the disease, very light episodic memory deficits are noticeable, along with deterioration in complex mental tracking and verbal fluency. Moreover, a short decline in functional performance in more complex tasks can be also perceived. In the following intermediate clinical stage, delayed recall of verbal and visuospatial / visuoconstructional information quickly deteriorates, while significant attentional and executive function decline also start to become more evident, with important alterations in several aspects of daily life functioning. As the disease progresses, cognitive impairment becomes broad and severe, with aphasia and apraxia (which may also appear earlier in the course of the disease) becoming prominent aspects in this later stage. Psychotic symptoms and profound alterations in behavior are also typical at this stage, and communication is severely affected. It becomes almost impossible to perform basic daily activities in an independent way and health condition deteriorates even more, eventually ending up in death (Albert, Moss, Tanzi & Jones, 2001; Abreu et al., 2005).

Acetyl-cholinesterase inhibitors are the core symptomatic treatment available, with proven beneficial effect at the mild to moderate stage, in different cognitive functions and in functional performance of daily activities (Birks, 2006).

Sociodemographic characteristics have been shown to affect cognitive performance in AD individuals. A higher educational level seems to be associated with an increase of synaptic density in cortical regions and with compensational strategies for cognitive deficits (Cummings, 2004). Subjects with more years of formal education seem to have a better performance in neuropsychological testing. Moreover, high

cognitive reserve is believed to alter the trajectory of cognitive performance by delaying the onset of cognitive symptoms of AD (Wilson et al., 2009).

In summary, the clinical diagnosis of AD relies on information from a variety of sources, under rigorous diagnostic guidelines. Such information includes patient and family history, a neurological examination, physiological and neuroimaging studies and laboratory assessments. Yet much of the diagnosis will ultimately rely on the quantitative pattern and qualitative characteristics of cognitive functioning (Albert, Moss, Tanzi & Jones, 2001).

2.7. Mild cognitive impairment

Early detection of AD has been a central topic in the scientific community and many researchers have dedicated their time studying the transitional state between normal ageing and AD, such as mild cognitive impairment (MCI; Petersen et al., 2001). This condition presents a prevalence in the general population of 6.7% for subjects aged 60-64 years, 8.4% for individuals aged 65-69, 14.8% for ages between 75-79 and 25.2% for those aged 8084 (Petersen et al. 2018). These individuals show a high risk of conversion to AD, with progression rates of 3-10% (community studies) and 10-15% (clinical settings), (Petersen et al., 2001; Farias et al., 2009). In a recent random-effects meta-analysis, individuals aged 65 or more had 14.9% higher risk or progressing to dementia after two years. After a period of 2-5 years, the relative risk of the diagnosis of AD was 3.0 (Petersen et al., 2018). Such variability is due to the heterogeneous definition and classification, with very few longitudinal studies trying to characterise its progression and conversion to dementia.

MCI definition has been constantly evolving throughout the years and became a wider concept with multiple clinical profiles and different aetiologies. The concept

rapidly moved from the research field into clinical settings, becoming a helpful aid in the diagnosis of dementia. Despite the general consensus around the relevance of MCI condition, this topic still generates great controversy amongst the scientific community regarding its definition, classification and clinical application (Chertkow et al., 2008). Nowadays, it is widely accepted that this is a heterogeneous condition, with some individuals reverting to a normal cognitive profile and others quickly progressing to different types of dementia. Some others seem to maintain the same level of cognitive deficit for several years.

MCI definition was first proposed by the Mayo Clinic in the 1990's, and the original criteria relied heavily on memory as the main cognitive function affected in the absence of an AD diagnosis. In addition, it required the presence of preserved abilities to perform daily life activities in an independent manner (Petersen, 1997; Petersen et al., 1999). A few years later in 2003, the criteria were broadened to include impairments in other cognitive domains, including clinical evidence of functional impairment. A group of researchers (Winblad et al., 2004) proposed new criteria to define MCI with the expression of concern regarding cognitive abilities from the patient / informant as the first feature to define MCI.

- Concern regarding a change in cognition (reported either by the patient or the informant);
- Impairment in one or more cognitive domains;
- Preservation of independence in functional abilities;
- Cognitive and functional performance not compatible with dementia.

A few aspects were considered fundamental to take into consideration when diagnosing MCI. Clinicians have to assess whether the cognitive complaint presented by the subject / informant corresponds to an objective measure of cognitive and

functional decline. This decline has to be tested using reliable instruments for diagnosis, such as clinical history, mental status examination and neuropsychological assessment. Cognitive abnormality has to be proved against reference standard deviation (SD) values, defined as 1 to 1.5 SD below the mean for their age and education matched peers (Grundman et al., 2004).

2.7.1. MCI classification

Recent studies have been trying to obtain more accurate data about this cognitive condition in order to predict progression to AD (Galimberti & Scarpini, 2012; Sperling et al, 2011). The complexity of this condition translates into a broad definition and a clinical application of great amplitude and over the years the need for a more comprehensive and accurate classification has been raised. The diagnostic criteria were expanded to encompass other aspects of cognitive decline apart from memory, that would help to better characterise disease progression and distinct clinical outcomes. Petersen and colleagues (2004) extended MCI definition and created four categories:

(a) amnestic MCI (aMCI): poor performance on memory tests;

(b) non-amnestic MCI (naMCI): poor performance on neuropsychological tests covering cognitive domains other than memory (language, executive functions, visuospatial abilities);

(c) single domain MCI (sdMCI): one single cognitive domain is affected;

(d) multiple domains MCI (mdMCI): two or more domains are affected;

This new classification allows four possible clinical subtypes: aMCI–single domain, aMCI–multiple domain, naMCI–single domain and naMCI–multiple domain.

In addition, it is possible to try to predict the type of dementia that MCI patients could develop (Petersen et al., 2004). Thus, the combination of clinical subtypes is of substantial help when predicting the type of dementia to which patients will evolve. aMCI subtype is more likely to develop AD, while naMCI forms often progress to fronto-temporal dementia, dementia with Lewy bodies and other non-AD dementia types. Multiple domain variants of MCI show a higher probability of progressing to AD, vascular dementia and depression.

The amnestic variant of MCI (aMCI) has been particularly studied given the increased risk of progression to AD and its first symptoms are episodic and semantic memory decline. (Petersen et al., 1999) Recent investigations have been questioning the more “traditional” early symptoms of MCI (episodic and semantic impairments) (Mitchell, 2008) and have been suggesting that early alterations in cognitive domains other than memory might be found in MCI, such as visuospatial decline (Drago et al., 2011) and executive impairments (Rozzini et al., 2007; Johns et al., 2012). This seems to trace a new path for the discovery of new cognitive markers of AD.

2.8. Executive functions

Executive functions (EF) represent those capacities that enable a person to engage successfully in independent, purposive, self-directed and self-serving behaviour (Lezak et al., 2004). Different theories about this concept refer to it as high order cognitive functions responsible for the regulation of lower cognitive operations. They comprise the capacity to control, plan, initiate and monitor a complex goal-directed behaviour (Stuss & Levine, 2002; Royal et al., 2002). This comprises different steps by which this behaviour is operated. The individual needs to understand the goal

and how to fulfil it. All the possible obstacles that might appear in the course of action have to be detected and have to be overcome. The plan must be initiated and the subject must continuously monitor the progress towards the goal, taking into account all necessary changes in strategy and correction of mistakes in order to achieve the goal. Finally, if the goal has been achieved, the individual has to judge his final action by comparing it to his initial goal before finishing the action (Perry & Hodges, 1999). This "umbrella term" includes several different complex cognitive processes and sub-processes such as planning, verbal reasoning, problem-solving, sustained attention, cognitive flexibility, inhibition, abstraction or working memory. Thus, this is not an unitary concept and the literature is yet to reach a consensus about its definition. Lezak et al (2004) defined four components: (1) volition; (2) planning and decision making; (3) purposive action; and (4) effective performance. Each involved a distinctive set of activity related behaviours. Diamond (2014) described three core EFs National Institute on Aging-Alzheimer's Association: inhibition (inhibitory control, self-control and interference control), working memory and cognitive flexibility. For this author, EF allow us to mentally play with ideas, quickly and flexibly adapt to changed circumstances, to consider what to do next, resist temptations, stay focused, and meet novel and unanticipated challenges. Executive functions influence social, emotional, intellectual and organisational aspects of one's life. All these executive components described above are essential to an effective performance of daily life's activities, when facing problems and making decisions.

The link between EF and frontal lobe activity has been extensively described in the literature (Godefroy, 2003; Elliot, 2003; Norman & Shallice, 1986; Duncan, 1986; Marsden, 1982). Several areas in the prefrontal cortex (integrative area of information coming from different cortical regions) seem to mediate EF. The anterior (prefrontal)

portion of the frontal lobes, comprising the dorsolateral, orbital / medial regions and dorsal anterior cingulate are usually associated with EF. Nonetheless, the prefrontal cortex has a straight connection to different brain regions, such as the basal ganglia (with striate structures, through thalamus and globus palidus) (Royal et al., 2002; Elliot, 2003).

With ageing, deficits in executive control become more frequent. However it is unlikely that ageing will influence each aspect of EF in the same way, therefore different types of deficits need to be addressed independently (Phillips & Henry, 2008)

2.9. Executive functions in Alzheimer's disease

It is only in recent years that studies have started to show that executive deficits are actually present early in Alzheimer's disease. In particular, deficits of inhibitory control (Amieva et al., 2004), attentional processes (Perry & Hodges, 1999) and visuospatial abilities (Landy et al., 2015) found in AD individuals are primarily due to prefrontal lobe degeneration (Salat et al., 2001).

EF are intrinsically connected to everyday tasks such as planning and cooking a meal, shopping or travelling to a new location. These common tasks represent major challenges to people with dementia early in the course of the disease. In patients with AD, impairments in divided and sustained attention, verbal fluency, working memory or in the ability to disengage and shift attention (Perry & Hodges, 1999; Green, 2000; Stokholm et al., 2006) hinder efficient performance of these tasks and are often a cause of distress.

A relevant amount of studies that focus on dysexecutive impairments in AD reveal a great methodological heterogeneity and lack consensus on the appropriate neuropsychological measures to be used. Thus, executive functioning is not usually

extensively assessed in all its sub-domains. It is not uncommon to observe one particular task being used to assess more than one aspect of EF or multiple versions of the experimental paradigm. Overall, it is undeniable the importance of a comprehensive neuropsychological evaluation to allow a clear delineation of the executive decline associated with Alzheimer's disease. This way, differences in performance in various EF tasks, which could reflect differences in the levels of deterioration of the various EF during the disease progression (Guarino et al., 2019). On the other hand, recent studies have been reporting early executive deficits prior to the diagnosis of dementia, parallel to memory impairments (Harrington et al., 2013). A longitudinal study of the progression to AD revealed changes in visuospatial abilities before memory decline, prior to dementia (Johnson et al., 2009). Schott and colleagues (2010) revealed that brain atrophy rates were correlated with low CSF Aß42 and impaired EF tests in the absence of a memory dysfunction in a study with older healthy controls. Another study found that specific measures of executive function, including inhibition, predicted cognitive decline in both normal controls and those with MCI or dementia (Clark et al., 2012). Altogether, these recent findings raise important concerns regarding subtle impairments in EF that remain undetected early in the course of AD, which could ultimately play a relevant role in better understanding the preclinical stages of the disease.

2.10. Executive functions in mild cognitive impairment

Although the decline in memory is the hallmark of patients with aMCI, recent findings have suggested that parallel executive deficits can be detected in these individuals. Some studies have found deficits in response inhibition, switching, cognitive flexibility and working memory alongside episodic memory alteration in MCI

patients, even in “pure amnestic” MCI (Johns et al., 2012; Brandt et al., 2009). Although the literature shows different positions regarding the relationship between the degree and frequency of EF impairments in MCI, alterations in inhibitory control seem to be frequently reported as one of the most affected sub-domains earlier in the course of AD (Johns et al., 2012; Hutchison et al., 2010). Other studies suggest that EF deficits precede dementia onset years before its diagnosis. Rapp and Reischies (2005) conducted a longitudinal study and found that attention and EF were good predictors of preclinical AD, discriminating in an effective way incident AD cases from nonconverters among a sample of MCI patients. Similarly, Harrington and colleagues (2013) observed a decrease in executive function (namely inhibition) in patients without memory impairments that were classified and preclinical AD patients (with abnormal beta amyloid$_{42}$/tau ratios). These findings seem to expand evidence that executive decline may appear in parallel or even before memory impairments in AD and might be good predictors of the conversion to the disease. However, it must be pointed out that the variability in the tasks used to examine different aspects of EF, and in the MCI classification, represent common limitations found in the literature nowadays.

Therefore, new paradigms and more sensitive tasks that could match the current constructs surrounding MCI classification and diagnosis might give an invaluable contribution to a more accurate assessment of EF decline in preclinical stages of AD.

2.11. Assessment of executive functions

Assessing executive functions is a topic that has been extensively explored by the scientific community throughout the years. The uncertainty around the taxonomy

and its multiple cognitive sub-domains raises the need for more complex and multifaceted measures that can tap a number of executive domains at the same time. The challenge for clinicians is how to accurately evaluate attentional processes, cognitive flexibility, planning or decision making within a structured and rigorous examination (Chan et al., 2008).

A brief review of the most commonly used tests when assessing executive functions is presented below.

- *Trail Making Test – version B* (Army Individual Test Battery, 1944): Version B assesses divided attention by asking the participant to connect sequentially and alternatively letters and numbers . It is also a reliable measure of visual search, scanning patterns, cognitive flexibility and information processing speed.
- *Wisconsin Card Sorting Test* (WCST; Grant & Berg, 1948): evaluates abstract reasoning and cognitive flexibility, as the individual is asked to change a specific strategy in response to an external contingency. The WCST consist of two sets of 64 cards, that vary in colour, shape and number of elements represented. Subjects are requires to sort cards according to one of these three dimensions. The task requires determining the correct sorting strategy based on the feedback given by the experimenter on the correctness of the performance. The sorting criterion changes suddenly without any warning and the participant must identifying the new appropriate strategy for sorting.
- *Stroop Test* (Stroop, 1935): this is one of the most used tasks to assess executive functions. this test measures cognitive flexibility, selective attention, inhibitory control and information processing speed. It includes 3 tests, two “congruous conditions” in which participants are required to name different color patches/blocks (C) and to read names of colors printed in black ink (W),

and a third condition, named color-word (CW) condition, where color-words are printed in an inconsistent color ink (the word 'pink' printed in green ink). The participant is asked to correctly name the color in 'C' condition, read the words in 'W' condition and name the color of the ink instead of reading the word. The reading / naming time is registered for each condition. A recent review, pointed out the Stroop test as the task that best discriminates between healthy and AD, amongst other widely used EF paradigms (Guarino et al., 2019).

- *Verbal Fluency test* (Benton & Hamsher, 1989): This test is widely used to measure executive function alterations. Verbal fluency facilitates information retrieval from memory. Successful retrieval requires executive control over cognitive processes such as selective attention, selective inhibition, mental set shifting, internal response generation, and self-monitoring (Lezak et al., 2004). The participant has to generate words beginning with particular letters (e.g. 'F', 'A', 'S') under a specific amount of time. There are many variants of the basic fluency test, namely category and action fluency tests. This task has demonstrated consistent results related to bilateral frontal lobe impairments (Robinson et al., 2012).
- *Iowa Gambling Task* (IGT; Bechara & Damasio, 1994): this is a decision-making task used to better understand the learning and choice processes underlying decision-making under uncertainty. The participant is asked to select a card from one of four sets of cards on each trial. The selection of the card will result in an associated reward, which can represent gains or losses. The goal is to maximise the gains and to minimise the losses. IGT has been proved to be a sensitive measure of decision-making impairments present in different neurological and psychiatric conditions. Patients with frontal lesions,

in particular in the ventromedial prefrontal cortex, lesions in the amygdala and the insular cortex have shown a poorer performance on the IGT (Bechara et al., 2007).

- *Rey-Osterrieth Complex Figure Test - copy test* (Rey, 1941): this test assesses visuo-spatial perception and visuo-constructional abilities, as well as problem solving. Participants are required to reproduce a complex line drawing by copying it freehand. Each drawing is scored for the accurate reproduction and placement of 18 specific design elements.
- *Wechsler Adult Intelligence Scale-Digit Span sub-test (backward version)*, (WAIS-R-BDS; Wechsler, 1981): In these tasks, a sequence of numbers is read by the experimenter and the participant is asked to recall the numbers in the reverse order. This is a reliable measure of working memory capacity.
- *Behavioural Assessment of the Dysexecutive Syndrome* (BADS; Wilson et al.,1996): This battery aims to assess deficits in cognitive flexibility, novel problem solving, planning, judgment and estimation and behavioural regulation with a set of different tasks. The literature reports its good ecological validity, as this battery measures executive functioning in more complex, real life situations and tries to predict problems that might happen in everyday life due to dysexecutive syndrome (Norris & Tate, 2000).

In sum, an extensive list of tests and batteries is available to assess EF in clinical and research settings. However, their efficacy and accuracy in detecting executive impairments in a comprehensive way is still a matter of debate. Some of the controversial issues rely on the fact that different studies use different tests to evaluate different EF sub-domains, or sometimes they use different versions of the same paradigm. Also, studies are not always consensual regarding the cognitive processes

that they engage. Despite the lack of consensus, the latest research over the past decades has been attempting to isolate specific cognitive processes of prefrontal functions and has created specific assessment tasks of executive functioning performance (Chan et al., 2008). This will hopefully contribute to a better understanding of EF and to a more accurate diagnosis of neurodegenerative disorders such as AD.

2.12. Eye movements and visual impairments in Alzheimer's disease and in mild cognitive impairment

Despite the well-documented impairment of episodic and semantic memory, recent findings have shown early deficits in other cognitive domains in AD, such as executive functions, attention and visuospatial abilities. Visual deficits may be related to both ventral and dorsal pathways (Rogers & Morrison, 1985). The distribution of neurofibrillary tangles and amyloid deposits in typical AD include the visual system, including the primary visual and association areas. In the dorsal visual pathway, there is a significant loss of long corticocortical projections from early visual areas to medial temporal areas (e.g., visual area V5). Greater impairment along the dorsal visual pathway in patients with mild AD result in abnormalities in 'lower' levels of visual processing such as colour perception, visual acuity, contrast sensitivity and motion perception, and also in 'higher' levels, such as visuospatial reasoning, face and object recognition (Bokde et al., 2009). Visual search is altered in AD and patients often have an increased difficulty detecting objects in both simple and complex scenes. This difficulty translates into deficits in recognizing salient features, an increased time to detect the target, and difficulty in shifting attention between global and local features (Parasuraman et al., 1995).

Neuroimaging studies suggested that AD patients have a reduced parietal activation and increased temporal activation in visuospatial processing, which leads to impairments in 'top-down' control during visual search (Pruvlovic et al., 2002). These visual deficits translate into impairments in eye movement behaviour, with AD patients showing altered reflexive and voluntary saccades, characterised by prolonged latencies, hypometria and decreased velocity. Saccade latency is prolonged and often correlates with the severity of the dementia (Yang et al., 2013; Garbutt et al., 2008). Smooth pursuit movements appear to be affected in a similar fashion, with increase latencies in the first saccades, decreased gain velocity and increased catch-up saccades (Garbutt et al., 2008). Mosimann and colleagues (2005) analysed visual exploration in AD patients during a clock-reading task. They observed that these subjects had different patterns of visual exploration, with a less focused exploration with fewer fixations inside the regions of interest (ROI), longer fixations in different areas and smaller saccade amplitudes.

Recently, the literature has been pointing out early visual impairments in MCI. Some studies have found memory impairments in MCI using eye movement metrics with novelty preference tasks (Yeung et al., 2013; Crutcher et al., 2009) where the number of fixations and fixation duration was related to short-term memory impairments and damages to the hippocampus in MCI subjects. On the other hand, while visuospatial deficits have been associated with cognitive decline typical of AD, is it still less clear to which extent visuospatial abilities are affected in MCI, specifically if there is a particular pattern of visual exploration associated to this condition. Recent findings suggest that top-down attentional control is affected in MCI, namely a reduction of attention shifting in visual search efficiency (Tales et al., 2005; Perry &

Hodges, 2003). However, little is known about the oculomotor metrics that best describe this decline in MCI patients.

2.13. Eye movements and executive functions in Alzheimer's disease and in mild cognitive impairment

As previously discussed in this chapter, deficits in executive function can be sub-divided into different features of EF, including planning, initiation, monitoring, inhibition and working memory. Voluntary eye movements have been linked to different anatomical circuitry and they can be used to characterise subtypes of EF within some clinical populations (Sereno et al., 2009). Saccades exhibit a wide range of features that vary quite dramatically from one to another. Saccade latency – usually around 150-200ms – represents the outcome measure and the culmination of a complex process of decision-making executed by neural structures (Carpenter, 1981). In consequence, distributional analysis of saccadic latencies has a wide range of applications in clinical settings and has been vastly used to study neurodegenerative diseases such as Parkinson's and Huntington's disease (Antoniades et al., 2010), Progressive Supranuclear Palsy (Lemos et al., 2017) and Alzheimer's disease (Heuer et al., 2013).

Two of the tasks that best emphasise top down control are the prosaccade (PS) and anti-saccade (AS), in particular the AS task (Hallett, 1978). In the PS task, subjects are asked to focus on a dot in the central visual field and then to fix their gaze to a target in the peripheral visual area. Latency is the time elapsed from the stimulus onset to the saccade onset. Task variations include changes in the location of the target and the time of stimulus onset (i.e. fixed or varying). In the AS task, the subject is requested to focus the gaze on a dot in the central visual field and then to look away

from the target, to its mirror position. This task involves two steps: 1) suppressing a reflex response to look at the target (PS) and 2) performing a voluntary action by purposely looking away from the target (AS). The AS task is a measure of executive functioning that is easily quantifiable and with good correlations with neuropsychological measures of EF. Also, it has been widely used to assess executive disfunction in several neurological and psychiatric disorders (Garbutt et al., 2008; Munoz & Everling, 2004). The AS task involves different cognitive processes such as inhibition, attentional control and working memory abilities (Munoz & Everling, 2004).

Healthy participants show some deficits in this task and frequently make prosaccades towards the target (errors). This error rate usually decreases with practice and is rapidly followed by a correct AS towards the mirror image location on the vast majority of trials (e.g. Tatler & Hutton, 2007). In comparison with PS, the latencies of correct AS are markedly increased—typically around 100ms longer than PS made to the same stimuli. Importantly, the latencies of erroneous PS towards the target are generally in the range of standard PS. Despite the considerable number of studies that have used the AS task, a significant variation in error rate and correct AS latency can be readily observed in healthy populations. Nonetheless the sources of this variation remain unclear (e.g. Coe & Munoz, 2017).

This simple paradigm has three variations: simple (step), gap and overlap paradigms. In the ‘Simple’ condition the fixation point offset coincides with the target onset. In the ‘Gap’ condition the central fixation stimulus disappears prior to target onset, usually with a 200ms gap. In the ‘Overlap’ condition, the fixation point remains on the screen during the target presentation usually for 200ms before it disappears. The gap paradigm facilitates the disengagement of attention from the fixation point and therefore allows faster saccadic response times, since there is no other visual

stimulus to compete with the target (Crawford et al., 2015). The ‘overlap’ paradigm slows the disengagement of attention since the persistence of the fixation point continues to capture attention whilst the target is presented. This is known as the ‘Gap Effect’ (Saslow, 1967) and it is calculated as the difference between ‘gap’ and ‘overlap’ saccade latencies. It is a measure of automatic saccade control, attentional disengagement and disinhibition in the superior colliculus due to the offset of the fixation point. These low-level neural mechanisms in the superior colliculus are not involved to the same extent in more volitional AS behaviours (Reuter-Lorenz et al., 1991). In the ‘overlap’ condition, visual attention is engaged and saccades are inhibited, resulting in slower latencies of saccades towards the stimulus. However, lower saccade latencies emerge in older compared to younger adults in the overlap condition. This may suggest a potential impairment of selective attention in ageing, due to an increased chance of attentional capture by peripheral stimuli (Noiret et al., 2017).

The difference between PS and AS saccade latencies (known as the ‘AS cost’) is a measure of the time needed to process an AS, i.e., the inhibition of an automatic saccade and the voluntary initiation of the correct AS. So far, no differences in the ‘Gap effect’ haven been found between younger and older adults, which may suggest that selective attention skills remain preserved with ageing. However, regarding the ‘AS Cost’ differences have been found in older adults reflecting the difficulties subjacent to higher cognitive demands such as goal maintenance and inhibitory control in the AS task (Noiret et al., 2017).

The current knowledge about the neural mechanism underlying PS and AS generation indicates the involvement of frontal regions in voluntary saccade control, specifically the dorsolateral prefrontal cortex (DLPFC), frontal eye fields (FEF) and

supplementary eye fields (SEF). While lesions in the DLPFC reflect impairments in saccade suppression needed when performing an AS, lesions in the posterior parietal cortex and supplementary motor area seem to affect PS latencies (Pierrot-Deseilligny et al., 1991; Munoz & Everling, 2004).

Eye movement performance in healthy ageing is still a matter of discussion in the current literature. Some evidence points to alterations in saccade latencies and increased direction error rates in healthy elderly subjects (Peltsch et al., 2011; Noiret et al., 2017), however some authors couldn't find significant differences between healthy older and younger adults (Eenshuistra et al., 2004). Despite this, the current literature seems to agree that automatic oculomotor parameters such as PS latencies seem to be minimally influenced by normal ageing. On the other hand, high-order cognitive aspects involved in voluntary saccades, such as inhibition of an automatic eye movement and initiation of voluntary AS are more prone to be affected by ageing. Oculomotor parameters produced during an AS task are associated with measures of EF, specifically with modified trails (attention measure), Stroop task (inhibitory control) and backward digit span (working memory; Mirsky et al., 2011).

Also, neuroanatomical structures such as the visual occipital cortex, parietal cortex, reticular formation and the superior colliculus responsible for the generation of PS, remain relatively preserved in elderly people. However, structures in the frontal and parietal cortices that are actively involved in AS performance, such as the right supplementary eye field and the left inferior frontal junction seem to be affected (Mirsky et al., 2011; Munoz & Everling, 2004).

Next, a more detailed description of the oculomotor measures associated to executive functioning in MCI and AD will be presented, specifically in inhibitory control, attention and working memory.

2.13.1. Inhibitory control

Inhibitory control is one of the most studied executive function abilities in AD due to the sensitivity of its cognitive tasks to early manifestations of the disease (Johns et al., 2012). Recently, several studies have used the AS task to study inhibitory control. The main feature of this paradigm is the ability to produce a behaviour measure of a) inhibitory control and b) implicit knowledge of the inhibition failure.

In AD, inhibitory control abilities show to be significantly impaired as revealed by the Stroop task results (Amieva et al. 2004). Patients with AD often reveal more errors in the AS, increased latencies and reduced corrective saccades (Kaufman et al., 2012; Garbutt et al., 2008). In fact, the increased number of errors in the AS task have been highly associated with a failure of inhibitory control in AD (Garbutt et al., 2008; Crawford et al., 2005). Both lesion and functional imaging evidence support a critical role of the DLPC and FEF in the AS task (Alichniewicz et al. 2013; Boxer et al. 2006). As discussed previously in this study, the AS task has a parallel nature involving a competition between the automatically triggered PS and the voluntary initiated AS (Munoz & Everling, 2004).

According to some authors, errors occur when inhibitory processes fail to stop the automatic PS towards the target, resulting in an increased likelihood of it reaching the threshold for saccade triggering. This account supports the idea of an active inhibitory mechanism as being critical to AS performance, which is not contingent on the interaction with a volitional saccade away from the target (Kaufman et al., 2012; Crawford et al., 2005). The theory of a competitive race between decision signals for the erroneous reflexive saccade and correct AS does, however, allow an alternative explanation. It is plausible to think that AS errors can also depend on a failure to sufficiently activate the correct response, as opposed to solely a failure to inhibit the

incorrect response (Eenshuistra et al., 2004). Thus, the literature is still not fully conclusive regarding the cognitive processes behind the AS task.

The number of studies about inhibitory control in MCI patients is still very modest, however the same pattern of inhibitory deficits appears to be present in this group. In a study conducted by Heuer and collaborators (2013), AD subjects exhibited a reduced number of AS compared to MCI and healthy control participants, with a lower rate of corrected errors. MCI individuals showed a similar performance to controls however the volumetry of frontoparietal region (typical of AD) correlated with AS performance. In all groups, AS correct answers correlated with executive measures in neuropsychological tasks, mostly with the Stroop task. In another study run by Alichniewicz and colleagues (2013), aMCI subjects were significantly impaired in the AS task, with a lower rate of correct AS compared to controls. Interestingly, reaction time didn't differ between groups. As in the previous study, significant inhibition impairments measured by the Stroop task were found in aMCI subjects. While no differences were found in cortex activation related to the PS task in aMCI and control subjects, aMCI exhibited a reduced bilateral activation of the frontal visual fields while performing the AS task.

2.13.2. Attention

Attentional abilities allow a selective and efficient allocation of limited neural resources towards a specific behaviour (Mazer et al., 2011). The use of eye movement paradigms in eye tracking studies may serve as an index of which aspects of a particular scene receive most attention (Hutton et al., 2008). Posterior parietal regions of the brain play an important role in visuospatial attention, and these include the

cingulate gyrus and the anterior frontal areas, in particular the frontal visual fields (Corbetta et al., 2008; Mesulam, 1981).

In a typical visual scene, different visual elements are selected using “bottom-up” or “top-down” processes. In “bottom-up” processing, the allocation of attention is dependent on the physical properties of the stimulus (e.g., colour, movement, shape) and it seems to be an automatic behaviour. On the other hand, “top-down” control of attention is directed to a specific object and determines the selection of a relevant visual element (Kastner & Ungerleider, 2000). The allocation of attention can be endogenously driven (volitional) and it is mostly used during visual search, when the subject intends to look for a certain object in a scene with different elements. AD patients evidence a higher frequency and longer duration of fixations while performing a visual search, as well as a reduced efficacy in adjusting attentional focus. This may indicate deficits in disengaging attention or in item processing. These patients also present difficulties in planning an organised and strategic visual search, which suggests an impairment of higher order processing of the allocation of attention (Tales et al., 2005; Rösler et al., 2005; Mosimann et al., 2004).

Saccadic eye movement has been vastly used as a tool to study impairments in attention (Leigh & Kennard, 2004). Neuroimaging evidence has shown that declines in PS are predicted by volumetric decreases in the parietal lobe (Garbutt et al., 2008), which is critical for visual attention (Posner & Petersen, 1990) and is affected by AD.

Moreover, controlled attention (the cognitive processes that redirect attention toward relevant information) play an important part in AS task performance. The executive attention allows maintaining task instruction and managing the potential conflict between two competitive responses, i.e., the erroneous PS and the correct AS (Engle & Kane, 2004). In the AS task, the peripheral target triggers the attention and

leads to a competition race between parallel PS and AS activations. If attention is preserved, the AS is quickly initiated, enabling the inhibition of the PS and the triggering of the correct AS. While it is known that attentional changes in AD affect this inhibition of the PS and the number of corrective saccades (Heuer et al.. 2013), little is known about the attention deficits in MCI. While recent findings (Yang et al., 2013) suggest that aMCI patients might present an increased difficulty in shifting their attention in the AS task, other authors fail to observe this change in attention in this group (Holden et al., 2018). Nonetheless, the extent to which attentional influences on AS performance can be separated from working memory (i.e., maintaining task instruction active) influences are not clear.

Divided attention also seems to be impaired in AD. As opposed to tasks with a single stimulus, in multiple-stimuli tasks divided attention is associated with an increased demand in cognitive processing in these patients (Perry & Hodges, 1999; Posner, 1978). In a similar way, a decline in divided attention can be observed prior to the establishment of AD. In a recent study, 50% of the MCI sample exhibited significant impairments in divided attention. This highly demanding cognitive process reflects early cortical impairments in MCI. In fact, in a study (Dannhauser et al., 2005) comparing MCI and elderly controls, both groups showed a similar activation of the left hemispheric prefrontal and extrastriate visual cortex. However, aMCI exhibited attenuated prefrontal activation and a slower processing speed compared with elderly controls. These findings reveal that prefrontal activation in divided attention tasks can be a potential incipient marker of cognitive decline in MCI.

2.13.3. Working memory

Working memory refers to the capacity to actively maintain and manipulate information in the mind. The working memory model proposed by Baddeley and Hitch proposes an executive control system with limited storage capacity. This model includes an attentional control system and two short-term storage systems, one for visual material (the visuo-spatial sketchpad) and one for verbal-accoustic material (the phonological loop). The model has a fourth component, the episodic buffer capable of integrating information from various components of working memory within a unitary system of episodic representations (Baddeley, 2005).

Previous findings revealed that, as in other EF measures, working memory is early impaired in AD. Oculomotor tasks are particularly adaptable to the study of visuospatial working memory. Working memory is a key factor in the control of AS, by maintaining the task instruction (i.e. look away from the target) when faced with competition from the prepotent stimulus (Mitchell et al., 2002). Thus, cognitive load in the working memory seems to affect performance in the AS task, namely in the ability to inhibit saccade triggering toward and rapidly executing a saccade opposite to the target (Crawford et al., 2013; Eenshuistra et al., 2004; Mitchell et al., 2002). Consequently, AD patients make fewer correct saccades and an increased number of errors, compared to age-matched healthy controls due to an inefficient executive-attention system in working memory (Noiret et al., 2018; Kaufman et al., 2012; Peltsch et al., 2014). Moreover, working memory impairment affects the correction of automatic erroneous saccades in AD. Crawford and collaborators (2013) found that subjects exhibit an increased difficulty in the AS task because the attention for tracking the location of the target competes with the requirement to also monitor and correctly recall the intended location of their eye movement.

Working memory deficits seems to facilitate the production of an increased number of anticipatory (express) saccades, i.e., saccades initiated prior to the onset of the target appearance, in the AS task (Noiret et al., 2018). However, the literature has failed to understand whether the working memory deficits in AD and in MCI have a significant impact on the frequency of anticipatory saccades (Alichniewicz et al., 2013; Crawford et al., 2005). Regarding working memory performance in MCI, other studies found the same impairments in maintaining and monitoring a mental representation (instruction) as a product of working memory. Although most of the studies are less informative regarding working memory impairments in MCI, some findings seem to identify working memory deficits while other executive functions remain relatively preserved. In a recent study, aMCI subjects required more time to implement an effective organizational strategy (Guild et al., 2014). The number of studies looking at the impact of working memory performance on saccadic movements is still very modest, and they are not consensual about the association between AS oculomotor metrics and neuropsychological measures of working memory in MCI samples (Holden et al., 2018; Peltsch et al., 2014) More studies addressing the impact of working memory deficits in the AS task in MCI subjects are needed, to better describe an incipient executive disfunction in the conversion to AD.

3. OBJECTIVES

3.1 Main objective

Characterise executive functioning in healthy control subjects, subjects with mild cognitive impairment and subjects with Alzheimer's disease through the analysis of the eye movement behaviour.

3.2. Specific objectives

- Identify executive functioning in healthy controls, subjects with MCI and subjects with AD.
- Define an oculomotor profile associated to executive functioning decline in healthy controls, subjects with MCI and subjects with AD.
- Determine the contribution of oculomotor measures for the distinction between healthy controls, subjects with MCI and subjects with AD.

4. HYPOTHESES

4.1. Hypothesis 1

Subjects with AD exhibit a greater executive dysfunction than healthy controls, with MCI subjects showing an intermediate impairment.

4.2. Hypothesis 2

The decline of executive functions in healthy controls, subjects with MCI and subjects with AD is associated to specific oculomotor patterns, namely with saccade frequencies and latencies. Specifically, oculomotor impairments in subjects with MCI and AD are associated with a decline in inhibitory control, attention and working memory.

4.3. Hypothesis 3

Oculomotor measures can separate between healthy controls, subjects with MCI and subjects with AD with a good accuracy.

5. METHODS

5.1. Setting

The study was carried out at the Psychogeriatric Unit (LIM-27) of Instituto de Psiquiatria / Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (IPq-HCFMUSP). This is a tertiary public psychiatric hospital in the state of São Paulo (Brazil) that provides a wide range of clinical services to the community, medical education and research services.

5.2. Ethical Aspects

The present study was reviewed and favourably approved by the Ethic Committee from Hospital das Clínicas da Faculdade de Medicina de São Paulo (CAAE: 63482415.9.0000.0068). All participants read and signed a written informed consent (Appendix A) stating volunteer participation, anonymous handling of data, safety guidelines and the accomplishment of good clinical practice at the local institution. Participants were also informed they were free to withdraw from the study at any time without any medical care or legal right being affected. A legal responsible caregiver was asked to sign the written informed consent in the case of AD participants. A copy of the inform consent form was given to all participants.

5.3. Participants

Participants were recruited between 2016 and 2018 from the psychogeriatric outpatient unit of LIM-27 (IPq-HCFMUSP) and from the local community, with support of media advertisements (TV, radio and social media). Additional recruitment was made electronically (e-mail) and by telephone, through the screening for potential cases and controls among older adults who manifested interest in participating in a

memory assessment program. After answering an initial questionnaire, eligible candidates were referred to a face-to-face interview with a member of the recruitment team, at the hospital outpatient unit, and enrolled to the cohort if inclusion/exclusion criteria were met. All participants of this cohort were assessed at baseline by a multidisciplinary team, and re-assessed annually (MCI and AD) or every two years if cognitively unimpaired (controls). All selected participants were initially screened at baseline by a team of neurologists, psychiatrists, neuropsychologists and speech therapists in order to collect initial information about their cognitive and clinical status. After this initial screening, participants were invited to complete the research protocol (clinical, neuropsychological and eye movement assessment). Diagnoses were established by multidisciplinary consensus taking into account clinical, neuropsychological, laboratorial and imaging data.

Subjects were finally included into one of three possible groups: cognitively unimpaired elders, irrespective of the presence of memory complaints (CTRL group); mild cognitive impairment (MCI group) or Alzheimer's disease (AD group). The final sample was composed by a total 93 subjects, being 28 controls, 44 with MCI and 21 with AD, all aged 60 years old and above.

5.4. Inclusion criteria

- Normal or corrected-to-normal vision.
- Aged 60 and above.
- Clinical diagnosis of MCI established according to the Mayo Clinic criteria (Petersen et al., 2004) with the severity of symptoms or consequent functional limitations insufficient to meet Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV-TR) criteria for Dementia.

- AD patients included diagnosis of possible or probable AD based on the DSM-IV-TR and the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA; McKhann et al., 1984) criteria.
- Healthy controls were assessed with the Montreal Cognitive Assessment (MoCA) screening tool (Nasreddine et al., 2005) and did not present any cognitive impairment. The corresponding inclusion criterion for the control group was a cut-off score above 26 in the MoCA.

5.5. Exclusion criteria

- Age below 60 years old.
- Illiteracy
- Neurological or Psychiatric conditions/events such as schizophrenia, bipolar disorder, major depressive disorder, traumatic brain injury, epilepsy, psychosis, current or recent evidences of alcohol, drug and/or other substances abuse, or other conditions assessed by structured interview.
- Ocular and/ or oculomotor impairments, such as glaucoma, cataract, visuomotor disturbance and insufficient or insufficiently corrected visual acuity.
- Moderate dementia or other types of dementia, confirmed by clinical / imaging evidences, with an aetiology other than AD.
- General inability to understand and carry out a computerized task.
- Calibration procedure problems or low percentage of eye movement recordings.

5.6. Diagnostic procedures

All selected participants were initially screened at baseline by a multidisciplinary team in order to collect initial information about their cognitive and clinical status. After this initial screening, participants were invited to complete the research protocol (clinical, neuropsychological and eye movement assessments).

A detailed clinical assessment was performed by a neurologist and a psychiatrist for diagnostic purposes only. All relevant medical and laboratory exams were requested, neuroimaging scans (CT and/or MRI) were obtained for all participants and additional tests/scans were requested on clinical basis. Some other measures of clinical / functional impairment were administered, such as:

- Mini Mental State Examination (Folstein et al., 1975);
- Montreal Cognitive Assessment (Nasreddine et al., 2005)
- Geriatric Depression Scale (Yesavage et al., 1983)
- Lawton Instrumental Activities of Daily Living (Lawton & Brody, 1969)

5.7. Neuropsychological assessment

All participants concluded an extensive battery of neuropsychological assessment, administered by a team of experienced neuropsychologists. The assessment was divided into two sessions (around one hour each), with the purpose of avoiding any possible fatigue that might affect cognitive performance. The following tests were administered:

- Rey Auditory Verbal Learning Test (Rey, 1964): this test is widely used to assess functions such as attention, memory, and learning ability in the auditory-verbal domain. A list of 15 words (list A) is read to the participant for 5

consecutive trials, and in the end of each trial the participant has to immediately retrieve as many words as possible. After these 5 attempts, a new list of 15 words is read to the subject (list B) who has to immediately recall as many words as possible (trial 6). After this last trial, the participant is asked to recall the words from list A without the examiner reading it again. After a 30 minutes delay time, the individual has to remember the words from list A (trial 7) without reading the list. Then the subject is submitted to a memory recognition test, in which a new list of words is read to the subject, comprising words from list A and B, but also 20 distracting words (phonologically and semantically similar to the words in list A and B). The task is to indicate if each word belongs to list A or not.

- Rey-Osterrieth Complex Figure Test - copy and delayed recall tests (Rey, 1941; Osterrieth, 1944): this test assesses visuo-spatial perception and visuo-constructional abilities, as well as long-term visual memory. Participants are required to reproduce a complex line drawing by copying it freehand (copy) and then drawing it from memory after a delayed period of time of 20-30 minutes (recall). Each drawing is scored for the accurate reproduction and placement of 18 specific design elements.
- Trail Making Test – versions A and B (Army Individual Test Battery, 1944): this is neuropsychological test of visual attention and cognitive flexibility. The participant is required to connect, as quickly as possible and in the numerical order, a sequence of numbers from 1 to 25, randomly distributed on a paper sheet. In version B, the participant is required to alternatively connect a sequence of numbers, from 1 to 13, and a sequence of letters, from A to L, in the correct numerical and alphabetical orders, as quickly as possible.

- Stroop Color-Word Test (Stroop, 1935): this test measures cognitive flexibility, selective attention, cognitive inhibition, and information processing speed. It includes 3 tests, two “congruous conditions” in which participants are required to name different colour patches/blocks (C) and to read names of colours printed in black ink (W), and a third condition, named colour-word (CW) condition, where colour-words are printed in an inconsistent colour ink (the word “rosa”, ‘pink’ in portuguese, printed in green ink). The participant is asked to correctly name the colour in C condition, read the words in W condition and name the colour of the ink instead of reading the word. The reading / naming time is registered for each condition.
- Controlled Oral Word Association Test (COWAT) - FAS (Spreen & Strauss, 1998; Benton & Hamsher, 1976): Verbal fluency facilitates information retrieval from memory. Successful retrieval requires executive control over cognitive processes such as selective attention, selective inhibition, mental set shifting, internal response generation, and self-monitoring (Lezak et al., 2004). The participant has to name as many words as possible beginning with the letters ‘F’, ‘A’, ’S’, during 60 seconds per letter. The inadmissible words are repetitions, proper names or words with different inflection sharing the same root.
- Wechsler Adult Intelligence Scale-Digit Span sub-test - forward and backward versions (WAIS-R; Weschler, 1997): Attention and working memory are measured with this test. In these tasks, a sequence of numbers is read by the experimenter. In the forward version (FDS), the participant is asked to recall the numbers in the same order and in the backward version (BDS) in the reversed order. The size of the number sequences progressively increases

throughout the task, starting with 2 digits and extending up to 8 digits. The task stops when the participant fails to correctly reproduce the number sequence.

5.8. Eye movement assessment

Eye movements were recorded using a Tobii® TX300 eye tracker device (Tobii Technology, Stockholm, Sweden) and Tobii Studio® software (offline eye movement analyser, Tobii Technology, Stockholm, Sweden). This technology includes a binocular eye tracking system that registers eye gaze positions, a screen to present visual stimuli and real-time processing algorithms to estimate visual scanning parameters and eye movement coordinates. The gaze angle is calculated by the relative position of the centre of the cornea and the pupil with an accuracy of ± 0.75°. Sampling frequency of the eye tracking system was 300Hz (300 gaze data per second). Participants were seated at a distance of approximately 64 cm from the 23' computer monitor (with a resolution of 1,920 x 1,080 pixels), where the stimuli were presented. During the test, subjects were allowed to move their heads freely and blink their eyes, which supported natural viewing of the visual stimuli.

In a pre-study phase of this project, a pilot protocol was created in order to test, edit and improve the design stage of the eye tracking protocol. A small sample of healthy control, MCI and AD individuals with a visual search task and eye movements were tested with an eye tracking equipment. Preliminary results from this study indicated that eye movement variables (fixation metrics) are useful for identifying visual search impairments in MCI and AD (Appendix B). Following the experience gathered from this study with eye tracking paradigms, an eye movement protocol was constructed for the present study, with Prosaccade (PS) and a Antisaccade (AS) tasks, each one with 'Simple', 'Gap' and 'Overlap' conditions.

In the Prosaccade PS task, participants were told to first fix their gaze on a red centre-screen fixation point (1° of visual angle) that appeared on a grey background (RGB: 128, 128, 128) for 1000ms. Then, they were instructed to look as quickly and as accurately as possible at a black target dot appearing either on the right or left side of the screen for 2000ms, with an eccentricity of ± 11° visual angle in the horizontal plane. As soon as the black target dot disappeared from the screen they were told to return their gaze to the red fixation point that indicated the beginning of a new trial, until the peripheral target dot appeared again. In the AS task, instructions were slightly different than in the PS task. Like in PS tasks, participants were instructed to first fixate their gaze on a central red fixation point until a target black dot appeared either on the right or left side of the screen. In AS tasks, participants had to direct their gaze as quickly and as accurately as possible in the opposite direction to the target dot location, i.e. looking away from the target. If they accidentally looked in the direction of the target, they were told to correct their eye movement by looking to the opposite side of the screen as quickly as possible. There were 3 conditions (simple, gap, and overlap) in both PS and AS tasks. In the 'simple' condition, the target appeared right after the central fixation point have disappeared from the screen. In the 'gap' condition, the fixation point disappeared 200ms before the target appearance. In the 'overlap' condition the central fixation point remained in the screen for 200ms after the target appearance (simultaneously with the target) and after that time, it would disappear.

5.9. Procedures

5.9.1. Experimental Sessions

The experiment was divided into two sessions. In the first session, participants took the neuropsychological and clinical assessments for about 120 minutes with an interval between both sessions. In the second session participants performed the eye movement for about 30 minutes. The recording session had a fixed task sequence: first the PS task, with the Simple, Gap and Overlap condition and after the AS task, with the conditions following the same order as in the previous task (Figure 1). All sessions began with the PS task to avoid any carry over effect from the AS task and to avoid confusion.

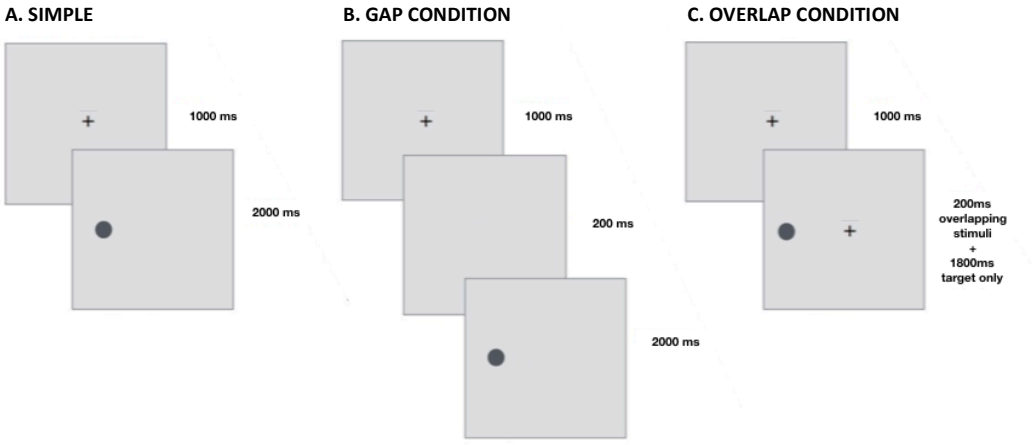

Figure 1. (A) Simple, (B) Gap and (3) Overlap conditions

For each condition (simple, gap, overlap), there were 12 trials with the target appearing pseudo-randomly in both directions (6 trials in both directions, left and right). In total participants performed 72 trials, 36 in each PS and AS tasks, with 1-2 minute interval between both tasks (Figure 2). Before each PS and AS task, a new calibration procedure was started and instructions were given to participants, both via the computer monitor and verbally by the experimenter. Then, 6 practice trials in each

condition (simple, gap, overlap) were performed to ensure that participants understood the upcoming task.

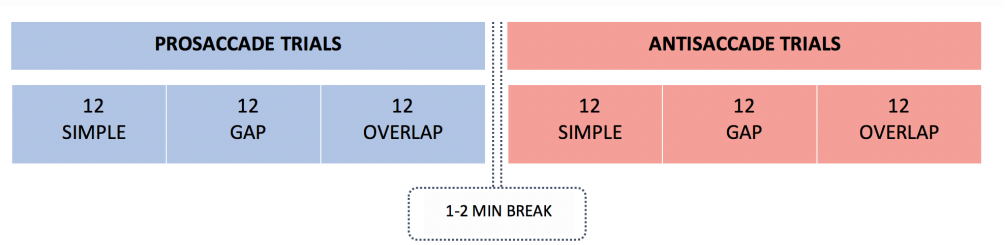

Figure 2. Sequence of trials presented in each experimental session.

5.9.2. Calibration

Each recording session started with a brief calibration test run by Tobii Studio® software to estimate the geometric characteristics of a subject's eye as the basis for an accurate gaze point calculation. Participants were asked to follow with their eyes a moving target that would navigate throughout 9 points on the screen. The resulting information was then integrated in the eye model and the gaze point for each image sample was calculated by the software. The calibration procedure was repeated as many times as needed, until eye movements were detected in each one of the 9 points and the quality of the calibration was assured (Figure 3).

A

B

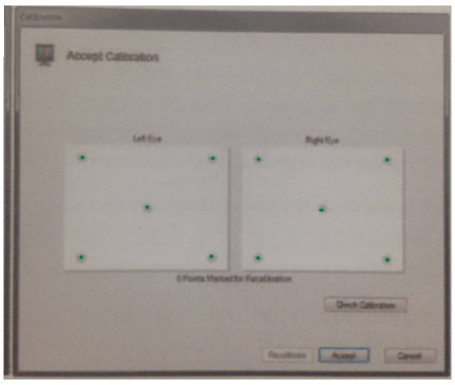

Figure 3. Calibration procedure examples (5 point calibration). A: Calibration procedure (5 points) with large errors. Error vectors are represented by green lines. The length and dispersion of each green line indicates the difference between the gaze point calculated by the eye tracker software and the actual dot position. Recalibration of certain points should be made if the lines are long or if there are no lines at all at a certain point. B: Ideal Calibration. Green dots represent the gaze point overlapping the actual dot position.

5.10. Data analyses

5.10.1. Neuropsychological tests analyses

Rey auditory verbal learning test (RAVLT): three scores were obtained separately: total, free recall and recognition. RAVLT - total is calculated by adding the number of correct words from the first 5 trials of immediate recall (word list A). RAVLT - recall is the number of correct words recalled (from word list A) after a 30 minutes delay. RAVLT - recognition is the number of correct words (from word list A) recalled among distracting words.

Verbal fluency test (FAS): inadmissible words (repetitions, proper names or words with different inflection sharing the same root) were eliminated from the analysis. Total score was calculated using the sum of all correct answers in each phonemic category ('F', 'A', 'S').

Trail Making Test – A and B: scores were obtained for each subtest using the total time (in seconds) spent to perform the task. The number of errors were not scored, however they translate into an increase of total time.

Stroop - Colours (C), Words (W) and Colours/Words (CW): scores were obtained taking into consideration the time spent (in seconds) to perform all three subtests. In the CW subtest, the number of errors was also scored.

Wechsler Adult Intelligence Scale-Digit Span sub-test (Forward and Backward versions): The total score was calculated for each version separately and corresponds to the number of correct answers. In the Forward version, the maximum score is 16 points and in the backward version is 14.

Rey-Osterrieth Complex Figure Test - copy and delayed recall tests: total scores for each test were calculated according to the manual guidelines (Spreen & Strauss, 1998). 18 particular characteristics of the figure were considered when

scoring. Each of the 18 items was evaluated according to a two-point scale. Two points were given when the item was placed and reproduced correctly; 1 point when the item was reproduced incompletely, placed incorrectly or presented some distortion; 0.5 point was attributed when the item was placed or reproduced poorly. A zero score was given when the item was absent or not recognized. The final score represented the total number of points made by the subject, representing a correct figure reproduction.

5.10.2. Eye movement data analyses

All data was initially processed using programing language on Matlab (all programming was performed by researchers DK and MP from the Visual Attention Lab at the University of Massachusetts, Boston, USA, collaborators in this project). Data sampling frequency is 300Hz and raw gaze positions were extracted per trial. Matlab allows the extraction in 1000hz (at each 1ms) of times and coordinated x and y of the gaze position (data journal). The software also allows the extraction of temporal markers of fixations and saccades.

Data extraction was performed in 3 stages, as presented in figure 4. Firstly, eye movement records were saved and processed for each participant after data collection. Secondly, during pre-processing stage the screen where all stimuli were presented was reconstructed, with the reconstruction and positioning of each stimulus and definition of all relevant areas for posterior analysis. Thus, the Matlab built-in function for detecting circle (imfindcircles) was used to compute the target centre and the radius.

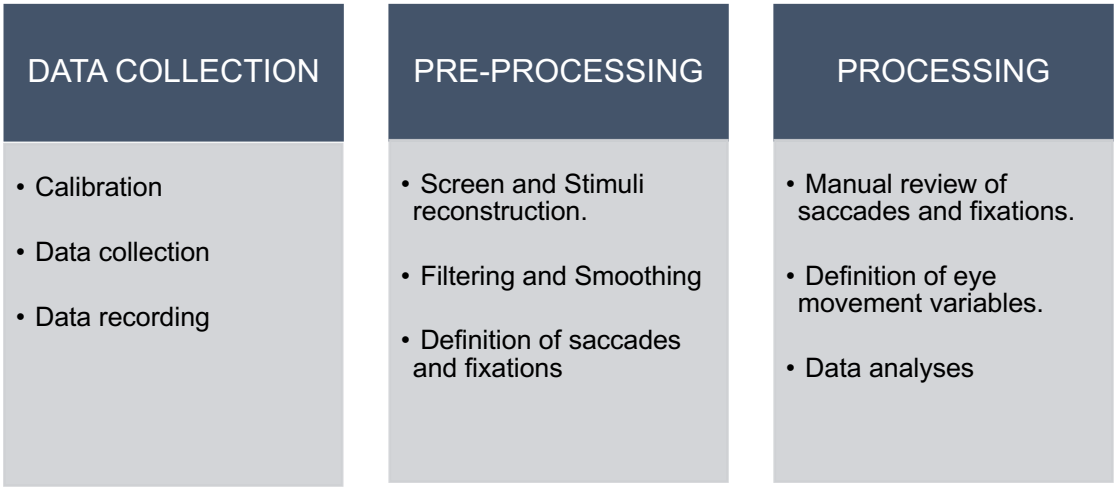

Figure 4. 3 stages of eye movement data collection and analyses.

Target centre location was extracted by colour thresholding method since the target and the background can be easily distinguishable by the colour and the intensity. A Kalman filter (I-KF, Komogortsev et al., 2010) was applied to the raw data, in which eye movement position signal was affected by noise due to the individual anatomical features and / or limited spatial resolution of the equipment. Eye movement variables were then identified based on the I-KF model (Figure 5).

Fixations and saccades were the two main eye movements to be considered in data extraction. Exceeding the threshold for automated saccade detection required an amplitude of 30 degrees/second. Fixation was defined as a point of gaze continually remaining within 1.5 degrees of visual angle for a period of at least 50ms. Eye movements were identified based on the I-KF model (Komogortsev et al., 2010). After fixations were classified, any consecutive fixations with a separation of maximally 1.5° visual angle and 100ms duration gap were merged into a single fixation.

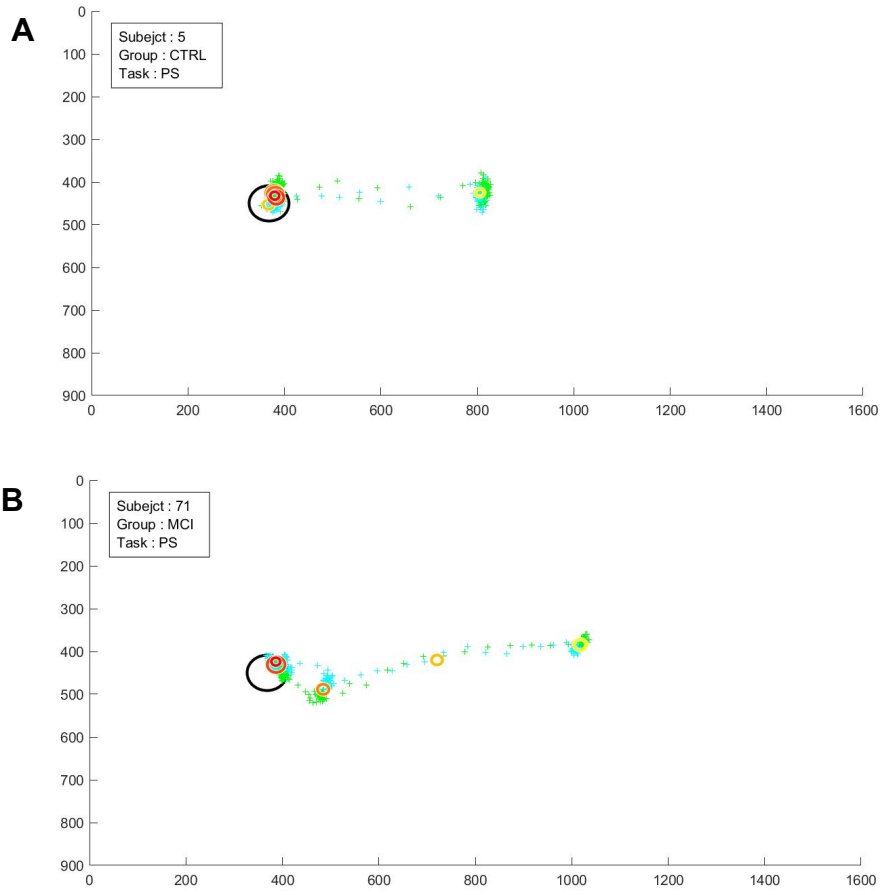

Figure 5. Example of gaze trajectory (left side of the screen, PS task) for a CTRL participant (A) and MCI participant (B). Fixation is represented as a coloured circle and its radius indicates durations. Temporal information of fixations is displayed as colour transitions from yellow to red. Gaze positions are scatter plotted and a black circle represents the target location in its actual size.

To evaluate the validity of raw gaze data, data loss was defined as the percentage of invalid gaze samples per trial. If the data loss was above 50%, the entire trial was ignored for the analysis. It is possible to have a small number of invalid gaze samples clustered during the trial due to eye blinks or looking away from the display region. Sometimes, the eye tracking software also failed to recognize the pupil in the scene due to other external artefacts. Therefore, an interpolation was performed to compensate invalid gaze samples with the dispersion criteria of 1 degree of visual angle. In other words, any invalid gaze sample within the maximum separation of 1°

of visual angle was interpolated. To compensate the noise, the algorithm required five consecutive gaze position samples exceeding the threshold in the same direction.

Thirdly, a large database was created containing all data organised by saccades and fixations for each subject. Each saccade should consist of its onset and offset locations, amplitude direction (horizontal left or right) and time stamps. For fixations, information about frequency and duration of each fixation with the correct sequencing order was also gathered. Relation between those features of eye movements with target locations can make us to define features (accuracy or correctness) for the analysis.

During processing stage, all data was manually revised by 2 experienced computational science researchers (DK, MP) and finally revised by the main researcher responsible for the study.

Saccades were considered for the analyses if they had a latency time equal to or greater than 100ms and less than 800ms. Saccades with a latency of less than 100ms were considered anticipatory saccades and saccades with a latency time greater than 800ms were considered delayed saccades.

Based on the final database, the following eye movement variables were derived:

- Percentage of correct saccades: Percentage of saccades with offset location on the target side with a proximity threshold of 3 degrees of visual angle (in the PS task) or in the opposite side of the target position (AS task).
- Percentage of errors: Percentage of saccades with offset located on the target side and with a proximity threshold of 3 degrees of visual angle (AS task).
- Percentage of corrected saccades: whenever the subject, after making an error (i.e. looking towards the target in the AS task) made a saccade in the opposite

direction (corrective saccade), by crossing the midpoint of the screen no later than 800ms (time to correct error threshold).

- Percentage of anticipatory saccades: Percentage of saccades directed toward the target (PS task) or away from the target (AS) before or <100ms after its appearance.
- Percentage of delayed saccades: Percentage of saccades directed toward the target (PS task) or away from the target (AS task) >800ms after its appearance.
- Latency of correct saccades: time measured (in milliseconds) from target onset until saccade onset towards (PS task) or away (AS task) the target.
- Latency of errors: time measured (in milliseconds) from target onset until the erroneous saccade onset.
- Latency of corrected saccades: time measured (in milliseconds) from the target onset until the onset of the second (corrective) saccade.
- Latency of anticipatory saccades
- Latency of delayed saccades

5.10.3. Statistical analyses

Different statistical analyses were run by use of SPSS (Statistical Package for Social Sciences, version 25.0), MATLAB and R softwares.

Sociodemographic and neuropsychological variables were compared between groups using one-way analyses of variance (ANOVA) models with the Brown-Forsythe test for multiple comparisons with unequal variance, and chi square tests (for categorical variables). Post-hoc tests (Tukey tests) were performed to determine the statistical significance of the differences between particular groups.

A three-way mixed design ANOVA (using age as a covariate) was used to compare different eye movement variables across groups. For all variables, the appropriate statistical corrections for heterogeneity (Levene test) and sphericity of variance (Greenhouse–Geisser) were made as needed. Group allocation (CTRL, MCI, AD) was the independent variable, and the within-subject factors were the experimental task (PS vs. AS) and the sub-task condition (Simple, Gap, Overlap). Values for right and left stimulus positions were not significantly different (paired t-test; $p > 0.05$), allowing the data to be collapsed across direction for each task (PS and AS). Post-hoc tests were performed to analyse between which groups the differences could be found.

Pearson's correlations between neuropsychological and eye movement variables for each task (PS and AS) were performed.

Additionally, it was intended to verify if eye movement variables were potential predictors of each of the neuropsychological variables. Multiple linear regressions were computed using stepwise method for the selection of eye movement variables, in order to select the best subset of predictors. The model aimed to eliminate redundant predictors, by removing variables one at the time until it is left only with the variables that explain the distribution the best.

Finally, a logistic regression (stepwise method) was conducted to identify the subset of eye movement variables that best differentiates between two groups (CTRL vs AD; MCI vs AD; CTRL vs MCI). ROC (Receiver Operating Characteristics) curves were plotted to assess the performance of the model. For all analyses, it was adopted a statistical significance of 5% ($p<.05$).

6. RESULTS

6. RESULTS

6.1. Description of sociodemographic data

As presented in table 2, 93 subjects were divided into 3 groups, 28 in the CTRL group, 44 in the MCI group and 21 were AD patients. Subjects were equally distributed regarding gender and age (p>.05). AD patients were found to have significantly less years of education than CTRL (p<.05) and MCI participants (p<.05).

Table 2. Sociodemographic data (mean/standard deviation values) for mild cognitive impairment (MCI), Alzheimer's disease (AD) and healthy control (CTRL) groups.

	CONTROLS	MCI	AD	K (df=2)	*p.*	Post-hoc test [(b)]		
						AD x MCI	CTRL x MCI	AD x CTRL
N (=93)	28	44	21					
AGE (years)	69.58 (5.71)	69.27 (6.61)	72.95 (5.71)	5.10	0.08			
GENDER f(m)	17.44 (12.79)	13.17 (4.89)	9.17 (6.44)	0.54	0.76[(a)]			
EDUCATION (years)	15.27 (3.44)	13.17 (4.89)	9.17 (6.44)	12.63	**0.001***	**0.0133***	(ns)	**0.002***

[(a)] Pearson's Chi-squared test; K = Kruskal-Wallis test was used as non-parametric statistical test; [(b)] Post hoc comparison used Bonferonni alpha adjustment. df = degrees of freedom; Significant values (p < 0.05) are marked with (*) and in bold; (ns) = non-significant

6.2. Neuropsychological profile of the three groups

Neuropsychological scores are summarised in table 3. Significant differences between the three groups were found in all neuropsychological scores (p<.05). As expected, AD patients performed worse on all tests than MCI patients, which in turn performed worse than control individuals, as expected.

After performing post-hoc tests (Tukey and Bonferroni tests for parametric and non-parametric data, respectively), it was possible to detect between which groups these differences could be found.

Table 3. Neuropsychologic test scores (mean/standard deviation) in controls (CTRLS), mild cognitive impairment (MCI) and Alzheimer's disease (AD) groups.

						Post-hoc test [(a)]		
	CONTROLS	**MCI**	**AD**	**F (df=2)**	***p.***	**AD x MCI**	**CTRL x MCI**	**AD x CTRL**
RAVLT total	46.77 (6.90)	34.54 (9.86)	26.78 (9.42)	186.533	***	***	***	***
RAVLT recall	10.04 (2.42)	6.44 (2.88)	2.22 (2.65)	291.985	***	***	***	***
RAVLT recognition	10.58 (5.37)	6.60 (5.31)	4.69 (7.76)	29.375	**	**	***	***
TMT - A	43.52 (14.88)	54.91 (24.16)	92.26 (43.70)	88.903	***	***	***	***
TMT - B	83.92 (24.11)	134.55 (88.91)	262.53 (143.83)	100.695	***	***	***	***
Stoop W (time)	16.77 (3.22)	20.60 (7.26)	23.93 (7.78)	44.052	**	***	***	***
Stroop C (time)	19.92 (3.30)	25.71 (8.92)	33.76 (11.51)	84.340	***	***	***	***
Stroop WC (time)	28.46 (8.90)	37.14 (12.83)	51.73 (19.64)	84.349	***	***	***	***
Stroop WC (errors)	1.27 (2.68)	1.68 (2.41)	3.63 (3.60)	23.597	**	***	ns	***
FDS	9.16 (2.12)	8.54 (2.70)	7.42 (1.54)	23.132	*	***	*	***
BDS	6.68 (1.31)	5.08 (1.84)	3.95 (2.20)	78.022	***	***	***	***
FAS	42.92 (8.63)	37.32 (10.91)	28.16 (13.36)	59.015	***	***	***	***
REY - copy	42.90 (41.25)	34.15 (16.10)	26.74 (10.39)	8.327	***	ns	**	***
REY - recall	21.42 (17.39)	14.99 (14.42)	6.93 (6.29)	20.709	***	*	***	***

RAVLT: Rey auditory verbal learning test; TMT: Trail Making Test; Stroop-W: Stroop-Word; Stroop-C: Stroop-Colour; Stroop-WC: Stroop-Word Colour; FDS: Forward Digit Span; BDS: Backward Digit Span; FAS: Verbal Fluency; REY: Rey-Osterrieth complex figure; F= one-way ANOVA with Brown-Forsythe test; (a) post-hoc comparison used Tukey test; df = degrees of freedom; significant values: *** p<.001; ** p<.01; * p<.05;

Memory scores (measured by RALVT total, recall and recognition scores), were significantly different across all three groups. Specifically, AD had a worse verbal memory performance than MCI patients, who performed worse than controls. The same trend can be observed between the three groups, when looking specifically to recognition scores.

In TMT (versions A and B; attention and executive function measures respectively) the same pattern was found, with CTRL's performing faster than MCI subjects (p.<.05), and AD participants being the slowest (p.<05).

Regarding the Stroop test (information processing speed, selective attention, cognitive flexibility and cognitive inhibition) all three groups scored significantly differently from each other in all three versions of the test (time in seconds for 'word', 'colour', 'word/colour' sub-tests). In the 'word' and 'colour' versions of the task, CTRL's reached the top score and AD's the worse score, with MCI participants showing an intermediate score (p<.05). In the 'word/colour' version, while AD had the slowest score of all three groups (M=51.73; SD=19.64), the MCI group was slower than the CTRL group (CTRL: M=37.14; SD=12.84; MCI: M=28.46; SD=8.90). When looking at the 'errors' score in the 'word/colour' task, CTRL group has the lowest number of errors, followed by MCI group and finally AD group, with the highest number of errors. Specifically, AD participants scored significantly worse than the rest of the groups (p<.05), which in turn performed in a similar way (p>.05).

In the Forward Digit Span test (attention test) and in the Backward Digit Span test (Working memory) once again all three groups performed differently from each other (p<.05), with AD group showing the lowest score and CTRL subjects having the highest score (subjects with MCI performed midway between the other groups).

Verbal fluency ('FAS' test) scores were again significantly different for all three groups (p<.05). AD's showed the lowest result, followed by MCI's and CTRL had the highest score in this test.

In the Rey-Osterrieth - copy test (visuospatial / visuoconstructional skills, attention, executive functions and working memory) CTRL group revealed a significantly lower score than MCI group (p<.05) and AD group (p<.05). However, both

patients groups (MCI and AD), performed similarly (p>.05). In the delayed recall test (visual memory), all groups performed differently from each other (p<.05), with AD subjects having the lowest score and CTRL subjects the highest score.

6.3. Differences between groups for each eye movement variable

In the next section, results will be presented as follow: first, ANOVAs indicating the differences between groups for each eye movement variable in both tasks (PS and AS; Table 4 describes mean and standard error values of each group for each task); second, the correlations between eye movement variables and neuropsychological measures for both tasks; third, linear regression analyses illustrating eye movement variables as potential predictors of each measure of executive function; fourth, multiple regression analyses (using ROC curves) determining the diagnostic value of eye movement variables in the classification of each sample group.

6.3.1 Percentage of correct saccades

ANOVA revealed a main effect of 'Task' ($F_{1,492=}$ 166.38; p<.001), where all subjects made more correct saccades on PS trials than on AS trials, and a main effect of 'Group' ($F_{2,492}$= 14.06; p<.001). In PS task, the interaction 'Task x Group' was not significant (p>.05), but it was significant in AS task ($F_{2,492}$= 13.62; p<.001). In this task, all three groups have a significant different percentage of correct saccades, with AD having the lowest percentage and CTRLs having the highest (Figure 6, A and B). No significant differences were found in the 'Subtask' condition, in both PS and AS task ($F_{2,492}$= 0.03; p=0.97).

6.3.2. Percentage of errors in saccades

Overall, groups performed significantly different from each other, as shown by the 'Group' effect in the ANOVA ($F_{2,492}$= 8.65; p<.001), with significantly more errors being made in AS than in PS, as demonstrated by the 'Task' effect ($F_{1,492}$= 60.84; p<.001). A main effect of 'Group x Task' interaction was found in the AS task (Figure 7, A and B; $F_{2,492}$= 8.12; p<.001), with AD subjects making significantly more errors than CTRL (p<.001) and MCI (p<.001) groups. MCI and CTRL groups made a similarly percentage of errors (p=.14). No differences were observed in PS task, where all groups barely made any errors (p>.05). No differences were found in the 'Subtask' condition ($F_{2,492}$= 0.26; p=.77), in both PS and AS task.

6.3.3. Percentage of corrected saccades

As observed in the previous variable, all three groups performed differently regarding the percentage of corrected saccades ($F_{2,492}$= 7.08; p<.001). Also, a main effect of 'Task' condition was observed ($F_{1,492}$= 125.94; p<.001), with a higher percentage of corrected saccades in AS task than in PS task. While there was not found a main effect of interaction 'Group x Task' for the PS task (p>.05), in the AS task all groups performed differently ($F_{2,492}$= 7.28; p<.001). AD subjects exhibited a higher percentage of corrected saccades than CTRLs, with MCI participants performing in an intermediate position (Figure 6, C and D). Once again, there was no main effect of 'subtask' condition ($F_{2,492}$= 0.14; p<.87) in both tasks.

Table 4. Mean (M), Standard error (St. Error) of eye movement variables in the prosaccade (PS) and anti-saccade (AS) tasks as a function of groups

		CTRL				MCI				AD			
		PS		AS		PS		AS		PS		AS	
		M	St. Error	M	St. Error	M	St. Error	M	St. Error	M	St. Error	M	St. Error
Correct (%)	Simple	100.00	0.00	89.55	3.13	99.61	0.27	81.37	3.70	98.75	0.91	67.94	5.34
	GAP	99.68	0.32	91.99	2.88	98.64	0.72	81.59	3.80	98.68	1.32	69.00	6.78
	Overlap	99.85	0.15	90.58	3.17	99.13	0.41	81.94	3.24	98.75	0.75	70.35	5.52
Corrected (%)	Simple	0.00	0.00	6.47	2.02	0.19	0.19	10.32	2.25	0.83	0.57	15.70	3.63
	GAP	.32	0.32	5.45	1.96	0.19	0.19	13.22	2.82	0.00	0.00	16.25	4.42
	Overlap	.15	0.15	6.28	2.65	0.19	0.14	11.16	2.70	0.42	0.29	17.94	3.70
Errors (%)	Simple	0.00	0.00	3.97	2.34	0.19	0.19	8.31	1.94	0.42	0.42	16.36	5.20
	GAP	0.00	0.00	2.56	1.24	1.16	0.70	5.19	1.45	1.32	1.32	14.75	5.31
	Overlap	0.00	0.00	3.14	1.11	0.68	0.36	6.90	1.66	0.83	0.65	11.71	5.33
Anticipatory (%)	Simple	2.78	1.18	4.49	1.24	6.09	1.20	5.99	1.16	3.75	1.54	9.39	2.00
	GAP	6.41	2.28	4.81	1.24	7.13	1.20	8.92	1.87	14.91	3.24	9.42	2.36
	Overlap	4.63	1.50	5.51	1.48	6.61	0.95	5.85	1.38	8.96	1.95	7.98	2.18
Delayed (%)	Simple	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.50	0.53	0.53
	GAP	0.00	0.00	0.00	0.00	0.19	0.19	.39	0.39	0.44	0.44	0.42	0.42
	Overlap	0.00	0.00	0.32	0.32	0.10	0.10	.48	0.35	0.46	0.32	1.97	0.92
Correct Latency (ms)	Simple	231.96	7.98	282.70	11.78	221.57	5.32	288.54	7.53	234.76	9.73	333.87	12.15
	GAP	218.15	7.86	282.76	12.41	210.87	6.07	282.14	6.79	215.83	10.01	342.87	16.91
	Overlap	225.65	7.36	312.20	11.57	216.22	5.18	321.76	8.88	226.32	8.72	365.08	17.06
Corrected Latency (ms)	Simple	0.00	0.00	159.37	42.51	6.05	6.05	219.66	37.92	49.60	35.83	309.17	58.93
	GAP	31.85	31.85	100.41	30.83	10.47	10.47	189.73	32.22	0.00	0.00	212.92	46.28
	Overlap	31.85	31.85	94.76	32.46	16.51	11.96	222.46	38.27	49.60	35.83	333.53	46.27
Error Latency (ms)	Simple	0.00	0.00	40.75	15.57	7.21	7.21	99.46	21.63	14.65	14.65	158.10	27.32
	GAP	0.00	0.00	38.27	17.07	21.13	13.52	73.96	18.45	9.33	9.33	125.46	22.95
	Overlap	0.00	0.00	66.02	24.15	28.34	15.08	112.40	25.17	23.98	16.95	123.86	34.07
Anticipatory Latency (ms)	Simple	6.90	3.01	19.33	5.77	22.70	4.60	19.05	3.82	13.51	5.63	31.51	7.58
	GAP	21.03	6.34	17.40	4.94	25.17	4.46	21.26	4.06	35.42	6.04	25.31	6.58
	Overlap	23.08	6.10	23.54	5.68	33.72	4.29	14.18	3.71	36.75	5.87	19.93	5.96
Delayed Latency (ms)	Simple	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	43.50	43.50	45.95	45.95
	GAP	0.00	0.00	0.00	0.00	19.44	19.44	22.01	22.01	45.45	45.45	43.00	43.00
	Overlap	0.00	0.00	38.78	38.78	19.44	19.44	38.77	27.10	88.95	61.24	191.85	88.11

6.3.4. Percentage of anticipatory saccades

ANOVA didn't reveal a main effect for 'Task' condition ($F_{1,492}$= 0.21; p=.65), indicating a similar amount of anticipatory saccades in both PS and AS tasks. However, a significant effect of 'Group' ($F_{2,492}$= 7.35; p<.001) and 'Subtask' ($F_{2,492}$= 4.86; p<.01) could be observed. All groups performed different from each other, with AD making significantly more (p<.01) anticipatory saccades than CTRL subjects, who had the lowest percentage of anticipatory saccades. MCI revealed a similar performance to CTRL and AD participants (Figure 7, C and D). For all groups, GAP subtask had significantly more anticipatory saccades (p<.05) than the other subtasks (SIMPLE and OVERLAP).

6.3.5. Percentage of delayed saccades

As in the anticipatory saccades, no main effect of 'Task' condition was found ($F_{1,492}$= 2.08, p=.15) or 'Subtask' ($F_{2,492}$= 1.39, p=.25) regarding delayed saccades. However, a significant effect for 'Group' condition was found ($F_{2,492}$= 5.74, p<.01). AD subjects have a poorer performance with significant more delayed saccades, which proved to be significantly different from CTRL (p<.001), who had the lowest number of delayed saccades. MCI had a similar performance to all the other groups (p>.05; Figure 7, E and F).

6.3.6. Latency of correct saccades

A main effect of 'Tasks' ($F_{1,492}$= 392.61, p<.001), 'Sub-task' ($F_{2,492}$= 6.77, p<.001) and 'Group' ($F_{2,492}$= 9.44, p<.001) was found for latency of correct saccades. In addition, a ''Group x Task'' ($F_{2,492}$= 9.91, p<.001) and "Task x Subtask" ($F_{2,492}$= 5.98, p<.01) interactions were observed. While no significant differences were found in PS

task between groups (p>.05), in the AS task AD participants had significantly increased saccade latencies (p<.001) than all the other groups (Figure 7, G and H). Specifically within the AS task, AD subjects revealed increased saccade latencies in the OVERLAP condition (p<.05) compared to both SIMPLE and GAP, where they had similar saccade latencies (p>.05).

6.3.7. Latency of Errors

A main effect of 'Task' ($F_{1,500}$= 77.45, p<.001) and 'Group x Task' interaction ($F_{2,500}$= 3.57, p<.05) was found for error latency. While in PS task all three groups performed in a statistically similar way (p>.05), in AS task CTRL group revealed a significantly shorter error latency than AD group (p<.01) with MCI participants performing similarly to CTRL and AD subjects (p>.05; Figure 8, C and D).

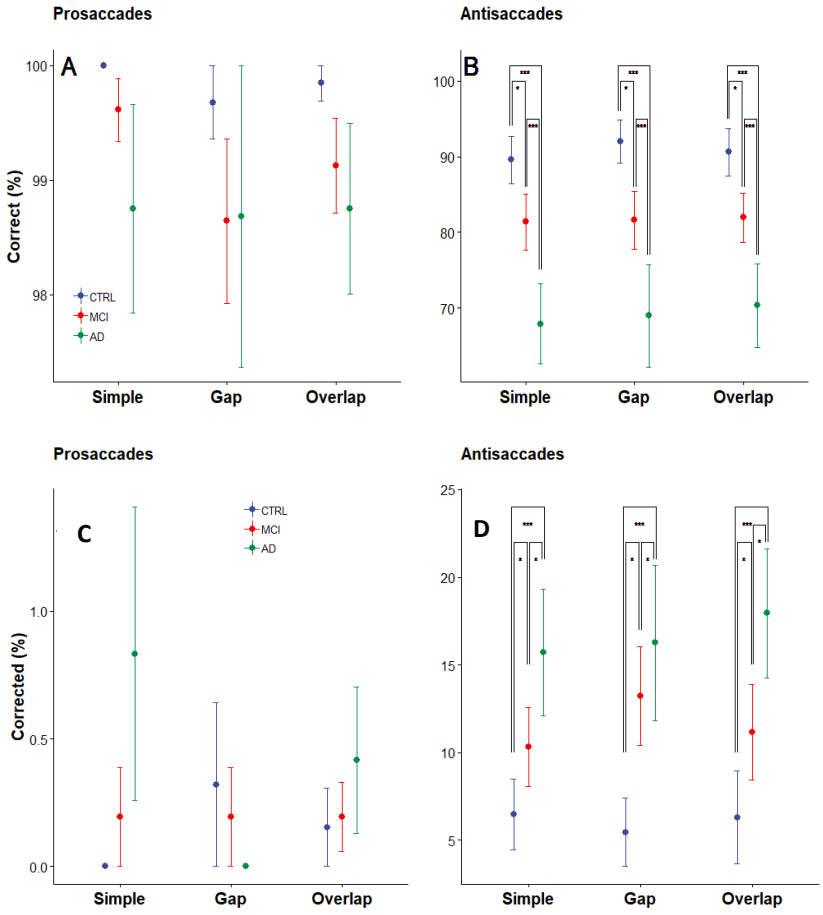

Figure 6. Percentage of correct (A, B) and corrected (C, D) in all three experimental groups, plotted by task (PS/AS) and condition (simple/gap/overlap); results include mean and error bars. * p-value < 0.05; ** p-value < 0.01 and *** p-value < 0.001.

6.3.8. Latency of corrected saccades

Once again, a main effect of 'Group' ($F_{2,500}$= 7.38, p<.001),'Task' ($F_{1,500}$= 140.35, p<.001) and 'Group x Task' interaction ($F_{2,500}$= 6.76, p<.001) was observed. AS task had significantly higher corrected saccades than PS task (p<.01). While no differences between groups were observed in the PS task, in the AS task both patient groups (MCI and AD) had significantly slower latencies in the corrected saccades than the CTRL group (p<.01; Figure 8, A and B).

6.3.9. Latency of anticipatory saccades

ANOVA revealed a main effect of 'Group' ($F_{2,500}$= 2.98, p<.05) and of the interactions 'Group x Task' ($F_{2,500}$= 0.43, p<.05) and 'Task x Subtask' ($F_{2,500}$= 5.49, p<.01). In the PS task, CTRL revealed significantly faster latencies (p<.05) in the anticipatory saccades than both MCI and AD groups (p>.05). Interestingly, in the AS task MCI participants had the lowest anticipation latencies and this was significantly different from CTRL's performance (p<.01). Anticipation latency in the AD group was, however, statistically similar to both CTRL and MCI groups (p>.05). Regarding the 'Task x Subtask' interaction, OVERLAP condition had significantly increased anticipation latencies compared to SIMPLE condition (p<.01) in the PS task. On the other hand, the SIMPLE condition had significantly faster anticipation latencies than the other sub-conditions in the AS task (p<.05; Figure 8, E and F).

6.3.10. Latency of delayed saccades

Although no main effects were found in 'Task' ($F_{1,500}$= 0.85, p=.36) and 'Subtask' ($F_{2,500}$= 2.88, p=.06) conditions, a main effect of 'Group' condition was observed (Figure 8, G and H; $F_{2,500}$= 8.95, p<.001). AD participants had significantly delayed latencies

(p<.01) compared to both CTRL and MCI groups, who performed similarly (p>.05). Although 'Subtask' condition didn't reach statistical significance, a tendency towards this significancy could be observed (p=.06). In 'SIMPLE' condition delayed latencies were significantly shorter when compared to 'OVERLAP' condition (p<.05) and 'GAP' evidenced similar delayed latencies to the other two conditions (p>.05).

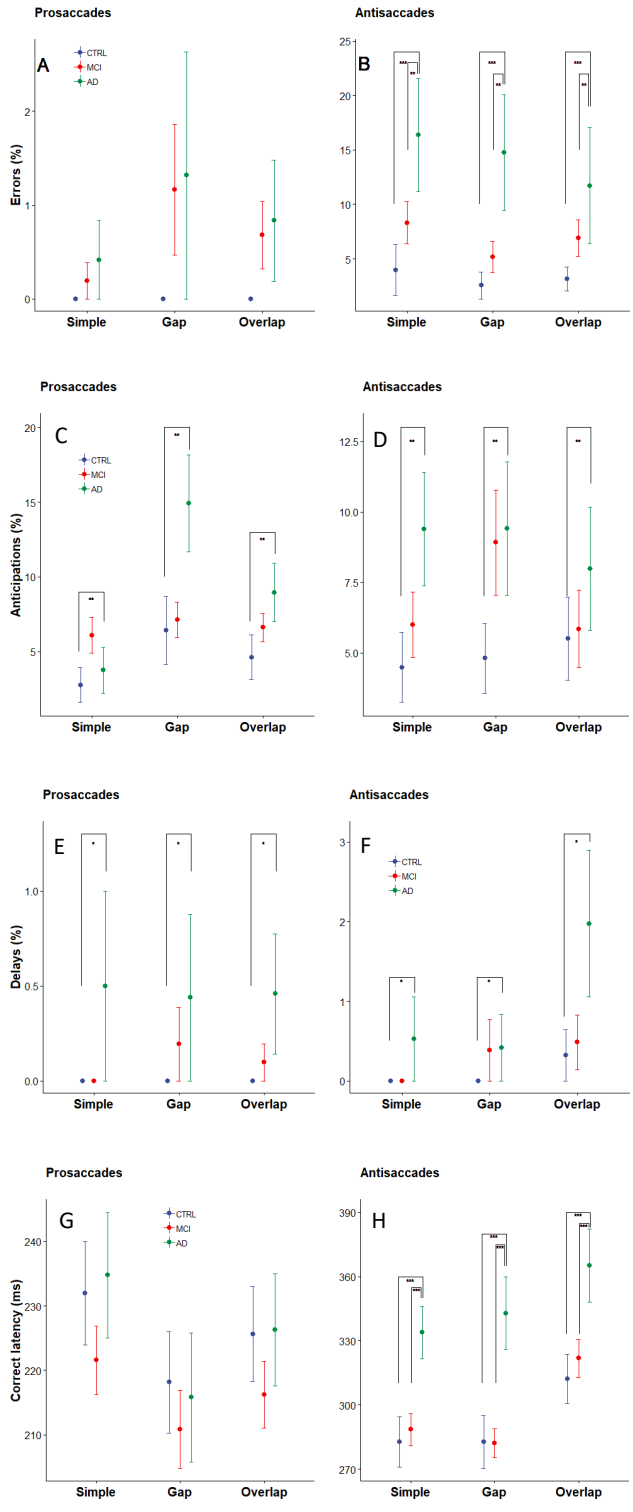

Figure 7. Percentage of errors (A, B) anticipatory (C, D) and delayed saccades (E, F) and latency of the correct saccades (G, H) in all three experimental groups, plotted by task (PS/AS) and condition (simple/gap/overlap); results include mean and error bars. * p-value < 0.05; ** p-value < 0.01 and *** p-value < 0.001.

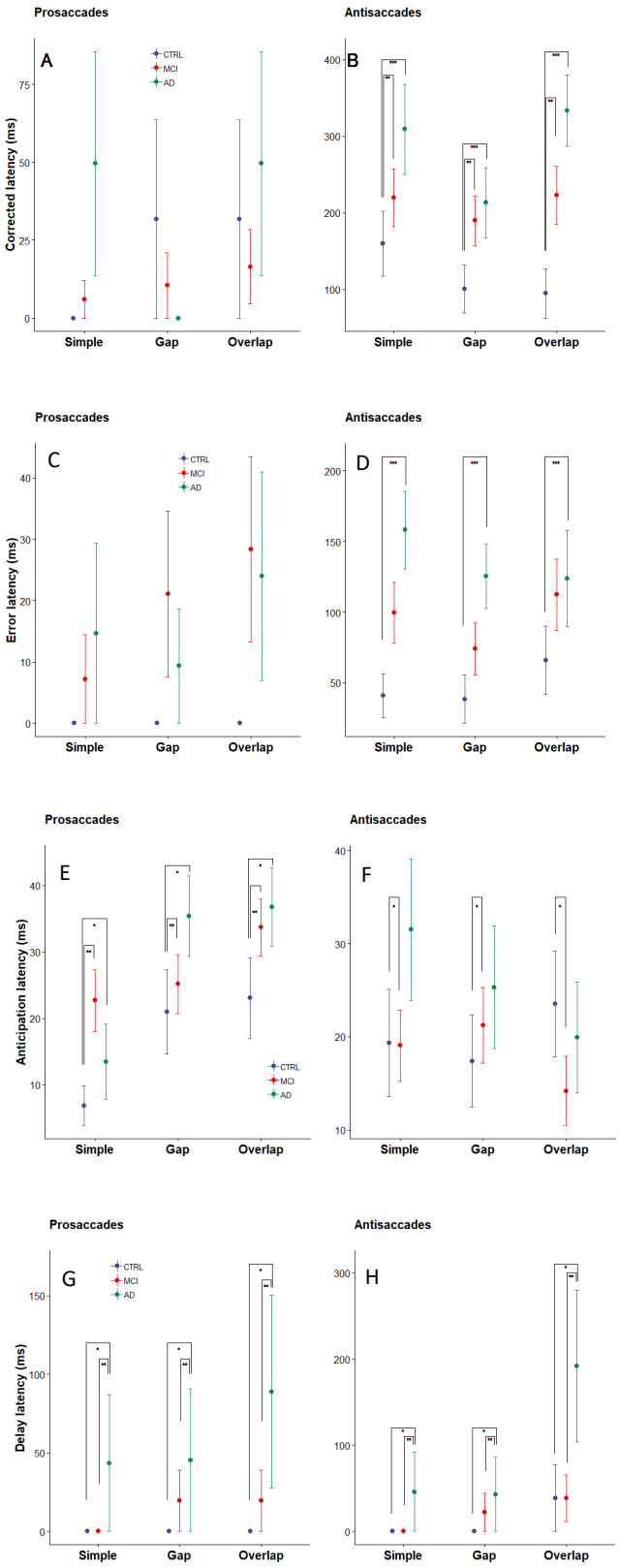

Figure 8. Latency of corrected saccade (A, B), errors (C, D), anticipatory (E, F) and delayed saccades (G, H) in all three experimental groups, plotted by task (PS/AS) and condition (simple/gap/overlap); results include mean and error bars. * p-value < 0.05; ** p-value < 0.01 and *** p-value < 0.001.

6.3.11. 'Gap effect' and 'AS cost'

The 'Gap effect' (Correct Latency$_{overlap}$ – Correct Latency$_{gap}$) was calculated for both tasks (PS and AS) and it is a measure of automatic saccade control. ANOVA results didn't reveal a main effect of 'Group' condition ($F_{2,487}$= 0.58, p>.05) or 'Task x Group' interaction ($F_{2,487}$= 1.52, p>.05). However 'Task' condition seemed to directly influence 'Gap effect' ($F_{1,487}$= 37.7296, p>.001), such that AS task produced a larger 'Gap effect' than PS task. Conversely, the 'AS cost' (Correct Latency$_{AS}$ - Correct Latency$_{PS}$) measures the voluntary saccade control. It was observed a main effect of 'Group' condition ($F_{2,475}$= 27.46, p<.001). All three groups performed significantly different (p<.05) from each other, with CTRL group having the shorter AS cost and AD having the larger AS cost (MCI group was in intermediate level of performance between CTRL and AD groups). The same trend was observed in 'Subtask' condition, and all three conditions had significantly different values (p<.05). As expected, the shorter AS cost was observed in the 'SIMPLE' and the larger AS cost was seen in the OVERLAP condition. OVERLAP revealed an increase of 24.63ms when compared to GAP condition and of 36.51 when compared to SIMPLE condition, as determined by linear regression analysis (Figure 9, A and B).

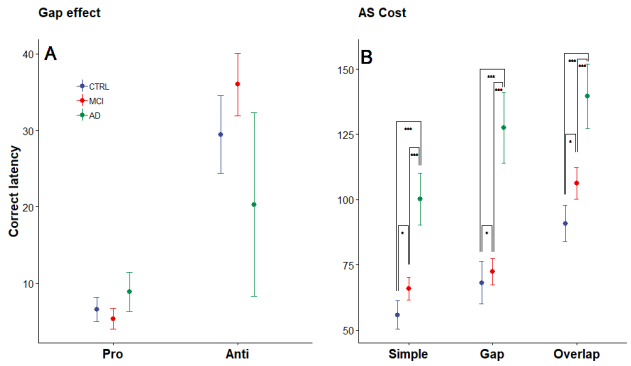

Figure 9. Mean gap-effect (A) and mean AS-cost (B) for each experimental group plotted by task (PS/AS) and condition (simple/gap/overlap); * p-value < 0.05, ** p-value < 0.01 and *** p-value < 0.001.

6.4. Correlations between neuropsychological tests and eye movement variables

In the present study one of the objectives established was to analyse the oculomotor profile associated with executive function impairments in MCI and AD patients. For that, it is relevant to determine if any correlation between eye movement variables and executive function measures can be established.

The relation between neuropsychological test scores and each eye movement variable was then tested for both PS and AS tasks and is presented in tables 5 and 6, respectively.

6.4.1. Prosaccade Task

All neuropsychological tests were correlated with the percentage of anticipatory saccades, excepting Digit Span and Rey-Osterrieth complex figure subtests. Inhibition, cognitive flexibility and attention measures (measured by times scores of Stoop test and FAS test) were correlated with the latency of anticipatory saccades. Additionally, latency of delayed saccades was also correlated with some measures of inhibition, cognitive flexibility (measured by Stroop WC-errors and FAS tests) and working memory (BDS sub-test). Working memory scores revealed significant correlations with several eye movement variables, namely percentages of correct (positive correlation), corrected, error and delayed saccades and also with latencies of corrected, error and delayed saccades. Also, significant correlations were found between latencies of correct saccades and both sub-tests of Rey-Osterrieth complex figure (measures of visuospatial / executive functions and delayed visual memory). Frequency of erroneous saccades (only in ‘Gap’ condition) also correlated positively with TMT-B version (measure of selective attention and cognitive flexibility).

Table 5. Correlations between eye movement variables (PS task) and neuropsychological tests

		RAVLT			TMT		Stroop				Digit Span		FAS	Rey	
		Total	Free recall	Cued recall	A	B	W	C	WC	WC (errors)	FDS	BDS		Copy	Recall
CORRECT	Simple	0.172	0.123	0.133	-0.018	0.121	-0.104	0.072	-0.074	0.043	0.096	**.303****	0.181	0.016	0.043
	Gap	0.155	0.051	0.167	-0.167	-0.206	-0.078	-0.079	-0.086	-0.136	0.122	0.209	-0.023	0.026	0.069
	Overlap	**.213***	0.100	0.207	-0.150	-0.109	-0.115	-0.034	-0.111	-0.098	0.150	**.322****	0.066	0.027	0.070
CORRECTED	Simple	-0.211	-0.149	-0.141	0.008	-0.104	0.106	-0.034	0.131	0.001	-0.176	**-.315****	-0.196	-0.013	-0.043
	Gap	0.061	0.156	0.030	-0.090	-0.094	-0.050	-0.077	-0.103	0.026	0.070	0.136	0.014	-0.026	-0.068
	Overlap	-0.127	-0.016	-0.092	-0.051	-0.142	0.051	-0.076	0.039	0.018	-0.093	-0.162	-0.146	-0.027	-0.074
ERRORS	Simple	-0.063	-0.046	-0.075	0.024	-0.097	0.063	-0.092	-0.022	-0.080	0.035	-0.178	-0.097	-0.009	.a
	Gap	-0.178	-0.100	-0.182	0.200	**.242***	0.096	0.105	0.120	0.132	-0.147	**-.258***	0.019	-0.019	-0.055
	Overlap	-0.192	-0.110	-0.200	0.198	0.191	0.111	0.073	0.112	0.106	-0.133	**-.302****	-0.011	-0.019	-0.051
ANTICIPATORY	Simple	-0.157	-0.140	**-.232***	-0.017	0.072	0.169	0.074	0.051	0.066	0.046	-0.135	**-.310****	-0.079	-0.114
	Gap	-0.141	**-.246***	-0.015	**.344****	**.262***	**.224***	**.249***	**.248***	0.070	-0.196	-0.164	**-.272***	-0.106	-0.182
	Overlap	-0.181	**-.246***	-0.127	**.229***	0.204	**.243***	0.212	0.211	0.086	-0.121	-0.188	**-.356****	-0.110	-0.180
DELAYED	Simple	**-.225***	-0.189	-0.026	0.077	0.073	0.140	0.138	0.153	**.377****	-0.069	**-.289****	**-.254***	-0.112	.a
	Gap	-0.068	-0.067	-0.061	-0.004	-0.174	0.086	-0.077	0.015	-0.054	-0.031	-0.060	-0.129	-0.027	-0.044
	Overlap	-0.200	-0.175	-0.065	0.047	-0.083	0.157	0.032	0.113	0.206	-0.069	**-.237***	**-.266***	-0.104	-0.042
CORRECT LATENCY	Simple	0.041	0.060	0.072	0.086	0.053	0.037	0.007	0.066	0.005	-0.203	0.055	-0.005	0.134	0.096
	Gap	0.137	0.165	0.148	-0.019	-0.039	-0.086	-0.133	-0.100	0.033	-0.118	0.149	0.070	**.283***	**.280***
	Overlap	0.102	0.122	0.127	0.038	0.020	-0.029	-0.068	-0.028	0.017	-0.170	0.120	0.040	0.224	0.197
CORRECTED LATENCY	Simple	-0.206	-0.145	-0.135	0.050	-0.128	0.155	-0.035	0.116	-0.024	-0.172	**-.313****	**-.255***	-0.012	-0.042
	Gap	0.085	0.179	0.069	-0.077	-0.085	-0.040	-0.077	-0.111	0.002	-0.004	0.125	0.001	-0.027	-0.065
	Overlap	-0.063	0.050	-0.030	-0.029	-0.148	0.066	-0.083	-0.015	-0.014	-0.112	-0.099	-0.160	-0.030	-0.078
ERROR LATENCY	Simple	-0.059	-0.045	-0.074	0.021	-0.094	0.058	-0.093	-0.023	-0.079	0.041	-0.174	-0.089	-0.009	.a
	Gap	-0.111	-0.028	-0.081	0.074	0.213	0.065	0.029	0.045	0.064	-0.169	**-.232***	-0.034	-0.013	-0.041
	Overlap	-0.127	-0.049	-0.111	0.074	0.123	0.088	-0.029	0.024	0.008	-0.117	**-.294****	-0.080	-0.015	-0.041
ANTICIPATION LATENCY	Simple	-0.118	-0.080	-0.204	-0.064	0.155	0.159	0.115	0.052	0.080	-0.092	-0.156	**-.255***	-0.070	-0.153
	Gap	-0.161	-0.207	-0.107	0.196	0.202	**.268***	**.362****	**.293****	0.110	-0.176	-0.136	-0.201	-0.132	-0.192
	Overlap	**-.219***	-0.206	-0.142	0.101	0.175	0.191	**.261***	0.210	0.078	-0.172	-0.136	-0.205	-0.149	-0.227
DELAYED LATENCY	Simple	**-.225***	-0.189	-0.026	0.077	0.073	0.140	0.138	0.153	**.377****	-0.069	**-.289****	**-.254***	-0.112	.a
	Gap	-0.075	-0.068	-0.065	0.002	-0.172	0.093	-0.075	0.018	-0.055	-0.037	-0.073	-0.140	-0.026	-0.042
	Overlap	-0.193	-0.166	-0.068	0.046	-0.099	0.158	0.019	0.104	0.174	-0.071	**-.229***	**-.263***	-0.100	-0.042

RAVLT: Rey auditory verbal learning test, TMT: Trail Making Test, W: Stroop-Word, C: Stroop-Colour, WC: Stroop-Word Colour, FDS: Forward Digit Span, BDS: Backward Digit Span, FAS: Verbal Fluency, Rey: Rey-Osterrieth complex figure; (.a) insufficient number of observations; values in bold: significant correlations; (*) p<.05; (**) p<.01

Table 6. Correlations between eye movement variables (AS task) and neuropsychological tests

		RAVLT			TMT		Stroop				Digit Span		FAS	Rey	
		Total	Free recall	Cued recall	A	B	W	C	WC	WC (errors)	FDS	BDS		Copy	Recall
CORRECT	Simple	**,378****	**,364****	**,246***	**-,373****	-0.185	**-,450****	**-,431****	**-,363****	-0.157	-0.025	0.128	0.200	0.155	**,274***
	Gap	**,347****	**,289****	**,232***	**-,294****	-0.159	**-,484****	**-,397****	**-,314****	**-,237***	0.139	**,368****	**,343****	0.165	0.233
	Overlap	**,286****	**,331****	**,244***	**-,232***	-0.159	**-,325****	**-,314****	-0.201	**-,225***	0.028	**,227***	0.180	0.171	**,283***
CORRECTED	Simple	**-,297****	-,278*	-0.127	0.195	0.117	**,370****	**,386****	**,315****	0.073	-0.046	-0.099	**-,259***	-0.153	**-,278***
	Gap	**-,255***	-0.212	-0.140	0.112	0.057	**,425****	**,308****	**,255***	0.078	-0.115	**-,327****	**-,340****	-0.159	-0.232
	Overlap	-0.125	-,231*	-0.017	0.096	0.127	**,305****	**,274***	0.069	0.016	-0.116	-0.192	**-,226***	-0.139	-0.246
ERRORS	Simple	**-,288****	**-,284****	**-,250***	**,373****	0.166	**,328****	**,286****	**,250***	0.166	0.077	-0.098	-0.062	-0.082	-0.177
	Gap	**-,304****	**-,252***	**-,240***	**,382****	0.210	**,339****	**,329****	**,245***	**,324****	-0.106	**-,254***	-0.193	-0.092	-0.128
	Overlap	**-,298****	**-,242***	**-,359****	**,246***	0.096	0.143	0.162	**,232***	**,334****	0.095	-0.126	-0.010	-0.106	-0.135
ANTICIPATORY	Simple	-0.092	-0.083	0.057	**,368****	**,379****	**,491****	**,366****	**,306****	0.094	-0.020	-0.085	**-,252***	-0.043	-0.128
	Gap	**-,270***	-0.180	-0.112	0.135	0.145	0.111	0.187	0.075	0.184	-0.127	**-,355****	-0.183	-0.106	-0.119
	Overlap	-0.100	-0.081	-0.112	0.215	**,359****	0.147	**,251***	0.181	0.171	0.054	-0.190	-0.136	-0.135	-0.166
DELAYED	Simple	-0.139	-0.077	-0.079	0.099	-0.150	0.189	-0.030	0.021	-0.077	-0.117	**-,235***	**-,274***	.a	.a
	Gap	-0.029	0.029	0.033	0.053	0.090	,245*	**,374****	0.125	-0.085	-0.030	-0.096	0.049	-0.055	-0.112
	Overlap	-0.033	0.017	-0.126	0.075	-0.042	0.066	0.111	0.046	0.034	-0.173	-0.197	-0.104	-0.067	-0.104
CORRECT LATENCY	Simple	**-,286****	-0.197	-0.115	**,228***	0.189	**,236***	**,318****	**,388****	0.195	**-,322****	-0.112	-0.179	-0.164	-0.263
	Gap	**-,268***	-0.207	-0.120	0.155	0.169	0.159	**,340****	**,316****	**,343****	**-,343****	-0.178	-0.195	-0.078	-0.162
	Overlap	**-,278***	-0.171	-0.014	0.128	0.080	**,274***	0.208	**,293****	-0.013	**-,246***	-0.213	-0.176	-0.089	-0.147
CORRECTED LATENCY	Simple	-0.155	-0.265	-0.290	0.304	0.294	0.065	-0.045	-0.059	0.199	-0.267	-0.144	**-,346***	-0.127	-0.318
	Gap	-0.163	-0.060	-0.272	0.065	0.042	0.277	0.273	0.145	0.038	-0.107	0.086	-0.144	0.091	-0.394
	Overlap	-0.080	0.016	0.091	0.132	0.053	**,441****	**,541****	0.285	0.067	-0.218	-0.144	-0.177	0.112	-0.247
ERROR LATENCY	Simple	-0.039	-0.066	-0.027	-0.064	-0.060	-0.286	-0.171	0.042	-0.022	0.003	0.122	0.143	0.346	-0.281
	Gap	0.239	0.153	0.214	-0.228	-0.130	-0.340	-0.280	-0.302	0.096	-0.168	0.274	0.229	0.248	-0.093
	Overlap	0.131	0.198	0.125	0.113	**,408***	-0.012	-0.077	-0.140	-0.038	-0.138	-0.130	-0.145	-0.174	-0.081
ANTICIPATION LATENCY	Simple	0.136	0.049	-0.021	-0.165	-0.014	0.028	0.061	-0.075	0.034	0.160	0.190	0.200	0.254	0.221
	Gap	0.167	0.129	0.155	0.090	-0.074	-0.180	0.056	0.035	-0.024	0.010	0.156	-0.035	0.100	0.144
	Overlap	0.105	0.181	-0.013	-0.116	-0.025	-0.139	-0.183	-0.175	-0.010	0.121	0.031	-0.028	-0.101	-0.005
DELAYED LATENCY	Simple	.a	.a	.a	.a	.a	.a	.a	.a	.a	.a	.a	.a	.a	.a
	Gap	.a	.a	.a	.a	.a	.a	.a	.a	.a	.a	.a	.a	.a	.a
	Overlap	0.070	-0.039	-0.222	0.013	0.201	-0.083	-0.089	0.275	0.053	-0.063	0.492	-0.228	-0.394	0.176

RAVLT: Rey auditory verbal learning test, TMT: Trail Making Test, W: Stroop-Word, C: Stroop-Colour, WC: Stroop-Word Colour, FDS: Forward Digit Span, BDS: Backward Digit Span, FAS: Verbal Fluency, Rey: Rey-Osterrieth complex figure; (.a) insufficient number of observations; values in bold: significant correlations; (*) p<.05; (**) p<.01

6.4.2. Anti-saccade Task

Trail Making Test version A (measures of processing speed and attention) correlated negatively with the percentage of corrected saccades and positively with the percentage of errors, percentage of anticipatory saccades ('Simple' condition) and with the latency of correct saccades ('Simple' condition). Version B (selective attention, cognitive flexibility and inhibition) correlated positively with the percentage of anticipatory saccades ('Simple' condition) and the latency of erroneous saccades ('Overlap' condition).

Stroop test (measures of inhibition, cognitive flexibility, processing speed and attention) exhibited correlations with all eye movement variables except with latencies of anticipatory, erroneous and delayed saccades. Specifically, Stroop W and C subtests were negatively correlated with the percentage of correct saccades and positively correlated with the percentage of corrected saccades, percentage of errors (only in 'Simple' and 'Gap' conditions), percentage of anticipatory saccades ('Simple' condition), latency of correct ('Simple' condition) and corrected saccades ('Overlap' condition). Additionally, Stroop 'W' subtest also showed a positive correlation with the latency of correct saccades (only in the 'Overlap condition) and the Stroop 'C' subtest was also positively correlated with the percentage of anticipatory (only in the 'Overlap' condition) and delayed saccades (only in 'Gap' condition). Stroop 'WC' subtest (cognitive flexibility and inhibitory control) was correlated with all eye movement variables except with the percentage of delayed saccades and the latency of corrected, anticipatory, erroneous and delayed saccades. A positive correlation was found with the percentage of errors and with the latency of correct saccades for all conditions ('Simple, 'Gap', 'Overlap'). This subtest was also positively correlated with the percentage of corrected ('Simple' and 'Gap' conditions only) and anticipatory (only

for the ‘Simple’ condition) saccades. A negative correlation was established with the percentage of correct saccades (‘Simple’ and ‘Gap’ conditions). When looking at the number of errors in the Stoop ‘WC’ subtest, a negative correlation was found with the percentage of correct saccades (‘Gap’ and ‘Overlap’ conditions) and positive correlations were established with the percentage of errors (‘Gap’ and ‘Overlap’ conditions) and with the latency of correct saccades (‘Gap’ condition).

Forward digit span (FDS) subtest (measure of attention) was negatively correlated with the latency of correct saccades.

Backward digit span (BDS) subtest (working memory measure) was positively correlated with the percentage of correct saccades (‘Gap’ and ‘Overlap’ conditions) and negatively correlated with the percentage of errors (‘Gap’ and ‘Overlap’ conditions) and with the latency of correct saccades (‘Gap’ condition).

FAS test (measure of self-monitoring, processing speed, inhibition and cognitive flexibility) was positively correlated with the percentage of correct saccades (only in ‘Gap’ condition). Negative correlations were found with the percentage of corrected saccades (all conditions), anticipatory, delayed saccades and with the latency of corrected saccades (‘Simple’ condition only).

Interestingly, significant correlations were between memory tests scores and some saccadic eye movements. The free and cued recall subtests (RAVLT) were positively correlated with the percentage of correct and erroneous saccade. RAVLT total score correlated with frequencies of correct (positive correlation), corrected, errors and anticipatory saccades and with the latency of correct saccades (negative correlations). Delayed recall subtest of Rey-Osterrieth complex figure test also was positively correlated with correct and corrected saccades. No correlation was found

between any variable of eye movements and the copy subtest of Rey-Osterrieth complex figure test.

6.5. Eye movement variables as predictors of executive functioning

A linear multiple regression with a backward selection was performed. Eye movement metrics were used as dependent variables, after controlling for group and age. This method considered all dependent variables together. Using the Akaike criterion (AIC), each variable was eliminated one at a time if it did not contribute to the regression equation and if it did not decrease the AIC value. Backward elimination stopped when deleting variables no longer improved the regression equation. It is worth mentioning that in some chosen models, some variables were not significant individually, however their deletion did not improve the equation. Nonetheless, they were kept in the model due to their combined contribution to the model. All significant p. values are highlighted in bold.

6.5.1. TMT-A

Altogether, Correct (%), Errors (%), Anticipations (%), Latency of correct saccades and latency of corrected saccades predicted 41.09% of TMT-A variance (R^2 = 0.4109, F=22.01, p<.001), as seen in table 7.

Table 7. Eye movement variables predictors of TMT-A

Eye movement variables	ß	Std. Error	t-value	p. value
Intercept	-90.738	22.597	-4.016	**<.001**
Correct (%)	0.33078	0.1625	2.036	**<.05**
Errors (%)	0.23487	0.2380	0.987	>.05
Anticipatory (%)	0.36115	0.176	2.054	**<.05**
Correct_latency	-0.04220	0.0265	-1.596	>.05
Corrected_latency	0.01563	0.010	1.543	>.05

6.5.2. TMT-B

The model (Table 8) selected Corrected (%), Errors (%) and Anticipations (%) as the best predictors of TMT-B, explaining 39.15% of the test variance (R^2= 0.3915, F=26.2, p<.001).

Table 8. Eye movement variables predictors of TMT-B

Eye movement variables	ß	Std. Error	t-value	p. value
Intercept	-120.1780	58.211	-2.065	**<.05***
Corrected (%)	-1.1293	0.418	-2.702	**<.01****
Errors (%)	-1.1407	0.554	-2.057	**<.05***
Anticipatory (%)	2.4013	0.625	3.844	**<.001*****

6.5.3. Stroop W-Time

Corrected (%), Errors(%), Anticipations (%), Delayed (%), Corrected Latency and Error Latency, altogether explain 29.46% of the Stroop W-Time variance (R^2= 0.2946, F= 11.19, p<.001), (Table 9).

Table 9. Eye movement variables predictors of Stroop W-Time

Eye movement variables	ß	Std. Error	t-value	p. value
Intercept	-5.281934	3.832	-1.378	>.05
Corrected (%)	0.039556	0.039	1.012	>.05
Errors (%)	0.110951	0.047	2.358	**<.05***
Anticipatory (%)	0.072489	0.041	1.756	>.05
Delayed (%)	0.541405	0.189	2.861	**<.001*****
Corrected Latency	0.001069	0.005	0.198	>.05
Error Latency	-0.008033	0.004	-2.257	**<.05***

6.5.4. Stroop C-Time

The model (Table 10) selected Correct (%), Anticipations (%), Delayed (%), Corrected latency, Error latency and Delayed latency as the best predictor of Stroop C-Time, explaining 37.26% of its variance (R2= 0.3726, F= 15.49, p<.001).

Table 10. Eye movement variables predictors of Stroop C-Time

Eye movement variables	ß	Std. Error	t-value	p. value
Intercept	3.594	6.448	0.557	>.05
Correct (%)	-0.053	0.040	-1.342	>.05
Anticipatory (%)	0.115	0.056	2.044	**<.05***
Delayed (%)	2.7250083	0.889	3.064	**<.01****
Corrected Latency (%)	0.0099647	0.007	1.366	>.05
Error Latency	-0.011	0.004	-2.452	**<.01****
Delayed Latency	-0.022	0.010	-2.256	**<.05***

6.5.5. Stroop WC-Time

38.05% of the variance of Stroop WC-Time can be explained by the following eye movement variables: Correct (%), Correct Latency and Error Latency (R^2= 0.3805, F= 25.98, p<.001), as shown in table 11.

Table 11. Eye movement variables predictors of Stroop WC-Time

Eye movement variables	ß	Std. Error	t-value	p. value
Intercept	-18.320	10.098	-1.814	>.05
Correct (%)	-0.097	0.04700	-2.062	**<.05***
Correct Latency	0.033	0.01379	2.418	**<.05***
Error Latency	-0.016	0.007	-2.368	**<.05***

6.5.6. Stroop WC-Errors

Corrected (%) and Errors (%) were the best predictors of the variance of Stroop WC-Errors (R^2= 0.115, F=7.34, p<.001), (Table 12).

Table 12. Eye movement variables predictors of Stroop WC-Errors

Eye movement variables	ß	Std. Error	t-value	p. value
Intercept	-3.611	1.840	-1.962	**<.05***
Corrected (%)	0.001	0.001	1.516	>.05
Errors (%)	0.003	0.001	1.936	**<.05***

6.5.7. FDS

Results (Table 13) suggested that Errors (%), Correct latency and Anticipation latency were the best predictors of FDS variance (R^2=0.1395, F=8.623, p<.001).

Table 13. Eye movement variables predictors of FDS

Eye movement variables	ß	Std. Error	t-value	p. value
Intercept	11.758	0.772	15.221	**<.001****
Errors (%)	0.034	0.015	2.340	**<.05***
Correct Latency	-0.010	0.002	-3.899	**<.001*****
Anticipatory Latency	0.008	0.005	1.579	>.05

6.5.8. BDS

The model selected Anticipations (%), Delayed (%), Corrected latency and Anticipation latency as the best predictors of BDS variance (R^2=0.2961, F=15.12, p<.001), (Table 14).

Table 14. Eye movement variables predictors of BDS

Eye movement variables	ß	Std. Error	t-value	p. value
Intercept	6.904048	0.228963	30.154	**<.001*****
Anticipatory (%)	-0.050	0.017	-3.022	**<.01****
Delayed (%)	-0.127	0.058	-2.193	**<.05***
Corrected Latency	0.001295	0.001682	0.770	>.05
Anticipatory Latency	0.012	0.005	2.263	**<.05**

6.5.9. FAS

As presented in table 15, altogether, Correct (%), Corrected (%), Anticipations (%) and Corrected latency explained 29.36% of FAS variance (R^2=0.2936, F=17.9, p<.00).

Table 15. Eye movement variables predictors of FAS

Eye movement variables	ß	Std. Error	t-value	p. value
Intercept	34.731	6.548	5.304	**<001*** **
Correct (%)	0.104	0.066	1.591	>.05
Corrected (%)	0.200	0.099	2.024	**<.05***
Anticipatory (%)	-0.153	0.073	-2.094	**<.05***
Corrected Latency	-0.010	0.004	-2.388	**<.05***

6.6. Diagnostic value of eye movement variables

In order to classify the groups according to their eye movement profile, a logistic regression was performed, comparing the groups with each other (CTRL vs. MCI, CTRL vs. AD, MCI vs. AD). Once again, 'backwards' method was used using 'step' function to define which variables would form the best model. The final model considered:

$$\log(Y) = \beta_0 + \beta_1 correct + \beta_2 corrected + \beta_3 errors + \beta_4 anticipatory + \beta_5 delayed + \beta_6 correctlatency + \beta_7 correctedlatency + \beta_8 errorlatency + \beta_9 anticipatorylatency + \beta_{10} delayedlatency + \varepsilon$$

The following model reveal the best distinction between CTRL and AD subjects (Table 16):

$$\log Y = \beta_0 + \beta_2 corrected + \beta_4 anticipatory + \beta_5 delayed + \beta_6 correctlatency + \beta_8 errorlatency + \varepsilon$$

Table 16. Logistic regression comparing CTRL vs. AD

Eye movement variables	ß	OR	Std. Error	OR (95%)	p. value
Intercept	-6.8971	0.00	1.67	0.00 - 0.03	**<.001**
Corrected (%)	0.0576	1.06	0.01	1.03 - 1.09	**<.001**
Anticipatory (%)	0.0520	1.05	0.01	1.02 - 1.09	**<.01**
Delayed (%)	0.3442	1.41	0.14	1.08 - 1.85	**<.05**
Correct Latency	0.0072	1.00	0.00	1.00 - 1.01	**<.05**
Error Latency	0.0052	1.00	0.00	1.00 - 1.01	**<.01**

Finally, a receiving operating characteristic (ROC) curve was used to assess the performance of the model. As presented in Figure 10, eye movement variables reveal a good accuracy when distinguishing between CTRL and AD subjects (AUC= 79.3%).

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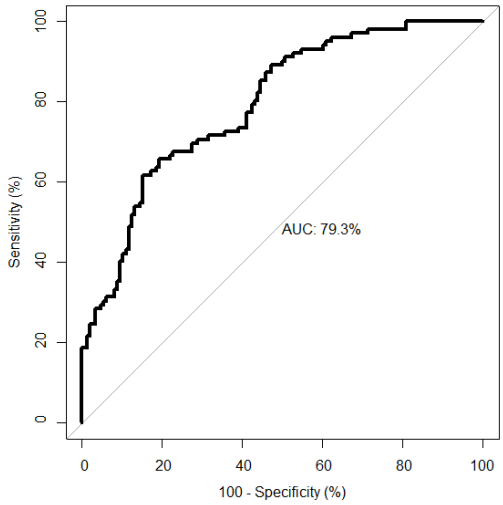

Figure 10. ROC curve with the best eye movement variables for differentiating AD from CTRL subjects

When separating MCI from CTRL individuals, Anticipatory (%), Corrected latency and Error latency showed the best performance (Table 17). ROC curve showed a moderate accuracy when separating between groups (AUC= 66.6%, Figure 11).

$$\log Y = \beta_0 + \beta_4 anticipatory + \beta_7 correctedlatency + \beta_8 errorlatency + \varepsilon$$

Table 17. Logistic regression comparing CTRL vs. MCI

Eye movement variables	ß	OR	Std. Error	OR (95%)	p. value
Intercept	4.6265	102.16	1.39	6.68 -1562.85	**<.001**
Anticipatory (%)	0.0235	1.02	0.01	1.00 - 1.05	>.05
Corrected Latency	0.0022	1.00	0.00	1.00 - 1.01	>.05
Error Latency	0.0036	1.00	0.00	1.00 - 1.01	**<.001**

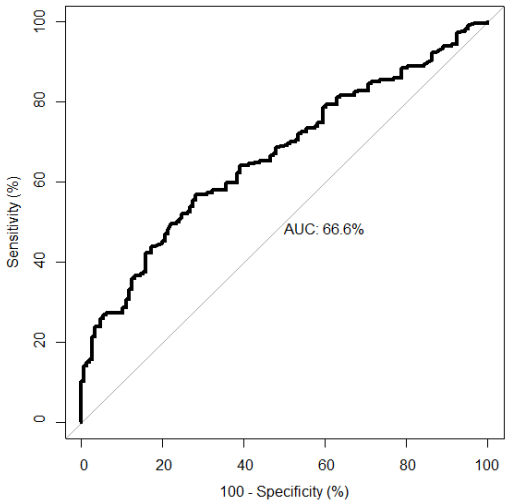

Figure 11. ROC curve with the best eye movement variables for differentiating MCI from CTRL subjects.

For the differentiation of MCI and AD subjects, the eye movements variables that best separated the groups were Corrected (%), Anticipatory (%), Delayed (%), Correct Latency and Delayed latency (Table 18).

$$\log(Y) = \beta_0 + \beta_2 corrected + \beta_4 anticipatory + \beta_5 delayed + \beta_6 correctlatency + \beta_{10} delaylatency + \varepsilon$$

Table 18. Logistic regression comparing MCI vs. AD

Eye movement variables	ß coefficient	OR	Std. Error	OR CI High	p. value
Intercept	-12.5167	0.00	1.90	0.00 - 0.00	**<.001**
Corrected (%)	0.0275	1.03	0.01	1.00 - 1.06	**<.05**
Anticipatory (%)	0.0269	1.03	0.01	1.00 - 1.06	**<.05**
Delayed (%)	-0.3833	0.68	0.24	0.42 - 1.09	>.05
Correct Latency	0.0124	1.01	0.00	1.01 - 1.02	**<.001**
Delayed Latency	0.0048	1.00	0.00	1.00 - 1.01	>.05

ROC curve (Figure 12) revealed a good accuracy in the distinction between the two groups (AUC= 77.9%).

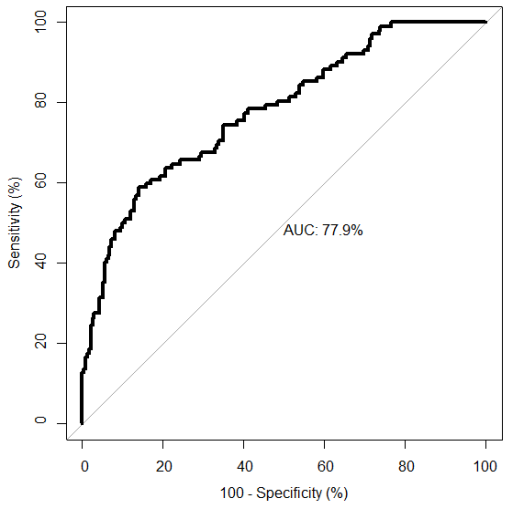

Figure 12. ROC curve with the best eye movement variables for differentiating MCI from AD subjects.

7. DISCUSSION

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7.1. Neuropsychological profile in healthy ageing, mild cognitive impairment and in Alzheimer's disease

One of the aims of the study was to investigate executive functioning performance in healthy control individuals, subjects with mild cognitive impairment and in subjects with Alzheimer's disease. As expected, all three groups exhibited a different performance in all cognitive domains assessed. Globally, AD showed a worse performance than CTRL individuals, with MCI having intermediate results between the other groups. All three groups performed differently, with MCI showing a slower rate acquisition that CTRL but faster than AD patients, as previously shown by the previous literature (Ribeiro et al., 2007; Estévez-Gonzalez et al., 2003).

Memory performance declines after a short-time delay and after a longer period of time, as seen in delayed recall and recognition scores with the three groups performing significantly differently. Semantic clustering strategies can be used during encoding phase and are known to benefit the retrieval phase (Denis et al., 1988). It can be argued that both patients groups have a poorer use of semantic strategies, which have a significant impact on memory recall and on delayed recognition deficits. When looking at the visual components of memory (Rey copy and delayed recall), once again all three groups had a different performance. Evidences from the literature confirm that MCI perform worse than controls in several visuospatial tasks and present a different patter of brain correlation between visuospatial abilities and grey matter values compared to healthy controls (Mitolo et al., 2013; Prvulovic et al., 2002).

Attention scores (measured by TMT- A/B and FDS) showed that patients with AD have a worse performance in sustained (TMT-A; FDS) and divided attention tests (TMT-B) than healthy older controls. MCI group showed intermediate scores between

CTRL and AD subjects. It can be argued that the intermediate decline in these attention tests observed in the MCI group can be due to the fact that FDS is measuring cognitive functions other than sustained attention, such as working memory capacity (Gagnon & Belleville, 2011). Divided attention also declined in MCI and in AD conditions, as observed in the present results. These deficits further declines in AD stage and they proved to go beyond the effects of age-related cognitive slowing (Johns et al., 2012; Rapp & Reischies et al., 2005).

Executive function measures (Stroop task, FAS, BDS) were significantly different between all groups in the present study and they seem to decline from healthy ageing to AD. A worsening of all measures of cognitive flexibility (Stroop, FAS), inhibitory control (Stroop, FAS), verbal fluency (FAS) and working memory (BDS) was observed in MCI condition, with a further decline in AD group. In the Stroop test, AD subjects responded slower and committed more errors than CTRLs, as a result of goal maintenance neglect. MCI subjects seem to be more impaired in terms of goal maintenance than CTRL subjects. However, they made a similar number of errors (interference face to incongruent trials) to CTRL subjects. It can be assumed that individuals with MCI slow down their responses facing trials that challenge their goal-maintenance abilities, allowing them to commit less errors and to maintain their task-specific goal. These results are consistent with previous studies (Belanger et al., 2010, Amieva et al., 2004) reporting that both normal ageing and MCI show deficits in resistance to interference and have a partial goal-maintenance impairment that is compensated for by reducing speed to prevent errors. Verbal fluency (FAS) was early impaired in MCI group compared to healthy controls and evidenced a further decline in AD group. This is consistent with other studies that showed a similar dysexecutive impairment in tasks that required phonemic fluency. This ability is known to be affected

in patients with impairments in dorsolateral prefrontal cortex or inferior frontal gyrus (Kim et al., 2016; Phelps et al., 1997). In working memory tests (BDS) an executive controlled attention is essential to manage the interference between processing and storage. Results from the present study corroborate other authors (Johns et al., 2012; Gagnon & Belleville, 2011), showing that working memory impairment in MCI was placed midway between that of controls and that of AD.

Finally, visuo-spatial / visuoconstructional abilities seem to be impaired in AD relatively preserved in MCI subjects, who performed similarly to healthy controls in this study. In normal ageing, perceptually-based tasks is relatively preserved compared to more active visuospatial tasks (Iachini et al., 2009). Thus, it can be argued that MCI are still relatively preserved in more basic visuo-spatial /visuo-constructional skills, besides evidencing dysexecutive deficits. However, this alterations in executive control seem to significantly impact MCI performance in the delayed recall condition of this task, showing impaired visual memory after a delay period of time.

Our results further support a key idea that executive functions can be useful in better describing cognitive decline previous to AD and that MCI patients frequently have mild deficits in cognitive domains other than episodic memory. Forlenza et al. (2009) uncovered that MCI patients with multi-modal impairments provide a more accurate prediction of conversion to AD. Emerging evidence support the idea that dysexecutive deficits may be present in aMCI individuals (Rozzini et al., 2007). Johns and colleagues (2012). The present MCI sample (multiple-domain MCI) exhibited prevalent deficits in executive sub-domains such as attention, inhibitory control (although with a relatively preserved goal-maintenance ability), working memory and verbal fluency. These deficits seem to further decline in AD, with the addition of impairments in visuo-spatial / visuo-constructional skills. Future studies should

consider the use of comprehensive neuropsychological measures of executive functions in order to thoroughly describe dysexecutive deficits and to better identify people at risk of progressing to AD.

7.2. Oculomotor profile in healthy ageing, mild cognitive impairment and in Alzheimer's disease

The present study compared oculomotor performance between healthy ageing controls, MCI and AD individuals in two paradigms: PS and AS tasks. It was observed that all three groups performed differently in both tasks, with the AS task better discriminating between groups than the PS task. Specifically, AS task proved to have a greater impact on the frequency and latency of eye movement variables in each group of participants.

In the automatic PS paradigm, most eye movement metrics could not distinguish between groups, such as the percentage and latency of correct, errors and corrected saccades. This in line with previous studies showing a similar performance in healthy controls and cognitively impairment individuals in their automatic oculomotor performance in the PS task (Shakespeare et al., 2015; Peltsch et al., 2014; Crawford et al., 2013; Abel et al., 2002). In fact, automatic saccadic parameters such as pro-saccade latencies are minimally influenced by ageing (Peltsch et al., 2011; Pratt et al., 2006), whereas more complex cognitive saccadic paradigms (such as the AS task) are more strongly influenced by ageing (Shafiq-Antonacci et al., 1999). One plausible explanation is that PS task is a simpler and less cognitive demanding task that becomes impaired later in AD, affecting visual occipital cortex, the brain steam oculomotor area, reticular formation and the superior colliculus (Tzekov & Mullan, 2014; Munoz & Everling, 2004). Other findings demonstrated, however, that latency

metrics in the PS task can separate between AD individuals from healthy controls (Heuer et al., 2013; Garbutt et al., 2008).

In this study, PS oculomotor metrics presented a wide variability within groups (particularly in latency metrics), which might contribute to the absence of differences between groups. Nevertheless, this intra-subject variability was also observed in previous studies (Shakespeare et al., 2015; Peltsch et al., 2014; Yang et al., 2011). Even after correcting for PS latencies (by performing a square-root transform of latencies and removing outliers), Shakespeare and colleagues (2015) could not find any differences in PS latencies between healthy controls and AD patients.

Altogether, these findings corroborate that PS metrics might not present enough sensitivity to detect cognitive decline. Moreover, recent findings showed that PS metrics tend to remain stable over time and AD patients showed no signs of deterioration in the PS task over a 12-month period (Crawford et al., 2015).

As previously described in the introduction chapter of this work, the AS task is a commonly used measure of cognitive inhibition, which requires suppression of a visually guided saccade toward a target and the initiation of a voluntary saccade in the opposite direction. Globally, the present results demonstrate that CTRL performed better than AD in the different eye movement variables analysed in this task, which is in line with previous findings (Crawford et al., 2013; Verheji et al. 2012; Boxer et al., 2012; Abel et al., 2002), with MCI subjects displaying intermediate levels of impairment between both of them. Regarding the frequency of this variables (% Correct, % Errors, % Corrected), all groups performed differently, with CTRL making more correct AS, less errors and less corrected saccades than AD and MCI performing intermediately. While MCI made a similar percentage of errors to CTRL, they performed significantly more corrected saccades than CTRL subjects and significantly less than AD subjects.

These results were consistent across all sub-tasks. This is consistent with previous findings using MCI and/or AD patients (Heuer et al., 2013; Mosimann et al., 2005), where both patients groups had a worse performance than CTRL group. Heuer and colleagues (2013) failed to find differences between MCI and CTRL in these variables, although MCI mean scores were numerically lower. The authors argued that this might have been a reflex of methodological issues (small sample size), which was corroborated by a strong correlation found between AD signature cortical thinning and AS performance in subjects with MCI.

When looking specifically to individual eye movement metrics, the frequency of errors in the AS task is frequently indicated as one of the more sensitive measures of executive disfunction in AS task (Noiret et al., 2017; Kaufmann et al., 2012; Boxer et al., 2012; Garbutt et al., 2008; Boxer et al., 2006). The present study found an increased number of AS errors in AD compared to CTRL group, corroborating the effectiveness of this metric. In addition, MCI demonstrated a percentage of errors similar to controls, suggesting that they might be relatively preserved in their ability to inhibit an erroneous automatic saccade (erroneous PS). In one of the very few studies that compared MCI and AD performances on the AS task (Peltsch et al., 2014), MCI and AD had a similar increased percentage of errors in the AS compared to healthy controls. This could be explained by the fact that the authors have included corrected saccades (erroneous PS followed by corrective saccade in the opposite direction to the target) in the percentage of errors. In the present study, the score for corrected saccades was analysed separately since it reflects other cognitive processes (working memory) besides inhibition, which seems to fail when a person commit an erroneous AS.

Latency metrics in the AS task were, once again, very informative of groups' performance, specifically in the MCI group. Similarly to previous studies (Heuer et al., 2013; Garbutt et al., 2008; Mosimann et al., 2005), CTRL subjects were faster initiating correct AS, making erroneous AS but also correcting errors than AD subjects. Despite being more efficient than patients when executing AS, elderly controls still registered increased latencies in the AS tasks comparing to the more automatic processing of PS tasks. Some authors have concluded that this could be explained by executive attention alterations in healthy ageing and not just simply by an age-related decline in processing speed (Noiret et al., 2016). MCI latency scores oscillated between the other groups. In the latency of correct saccades, MCI had faster latencies similar to CTRL ($p>.05$). Regarding the latency of errors, MCI performed midway between the other groups and could not be distinguished from them, which corroborates results from other authors (Heuer et al., 2013). However, in the latency of corrected saccades, MCI seem to be slower when correcting erroneous saccades, with a comparable performance to AD subjects. One can argue that, despite making less corrected saccades than AD (possibly due to a lower percentage of errors than AD), MCI subjects present a similar slower latency when correcting erroneous saccades to AD due to executive impairments. It can be suggested that MCI and AD patients exhibited a deficit in inhibiting an automatic saccade and in initiating a corrective volitional saccade during corrected saccades. In other words, it is suggested that a higher demand of cognitive resources in the generation of a corrected saccade impacts its latency significantly in both patient groups.

Interestingly, a 'sub-task' effect was observed and increased AS latencies were observed in the 'overlap' condition compared to both 'simple' and 'gap' conditions. If selective attention is less efficient, the 'overlap' condition should lead to reduced

attention to the central fixation point and to a faster attentional capture of the peripheral target (Noiret et al., 2016). In this study, AD subjects showed increased latencies in the overlap condition than the other groups. This can possibly be explained by a deficit in disengaging attention from the central fixation point during this overlapping period of time.

An interesting finding in the PS / AS tasks was the differences observed between groups in the anticipatory and delayed saccades. These two variables are very rarely considered in eye movement studies and are often excluded from the analyses. However, it is interesting to understand if they can bring more insight about top-down control on eye movement behaviour, given that they reflect a failure of active fixation in the upcoming need to make a saccade. Previous studies failed to find differences in these two variables between AD and CTRL subjects (Alichniewicz et al., 2013; Crawford et al., 2005). In the present study, AD subjects showed a higher frequency of both anticipatory and delayed saccades than CTRL subjects in both tasks and in all sub-tasks (Simple, Gap, Overlap). This result is corroborated by a previous study proving a higher number of anticipatory saccades in dementia (Hotson & Steinke, 1988). The increased frequencies of anticipatory saccades in MCI or AD cannot, however, be explained simply by the effect of ageing. In the present study healthy control subjects exhibited a reduced number of anticipatory saccades (around 5%). Furthermore, previous studies found a similar number of anticipatory saccades in both younger and elderly populations, proving that ageing alone does not explain a higher number of anticipatory saccades (Peltsch et al., 2011).

MCI subjects had an intermediate frequency of anticipatory and delayed saccades between the other groups and they could not be distinguished from them. Moreover, an effect of ‘sub-task’ was observed in all groups, with more anticipatory

saccades being made in the ‘gap’ sub-task than in the other sub-tasks, which is in line with the previous literature (Yang et al., 2011; Leigh & Kennard, 2004). It is known that in the ‘gap’ condition, attention is disengaged from any stimulus during that time interval, which facilitates the initiation of anticipatory saccades.

In fact, saccadic performance requires a balance between looking to a current point in space and redirecting gaze to the upcoming target. This competition is mediated by the superior colliculus (SC) or areas in parietal cortex, known to be affected in AD (Leigh & Kennard, 2004). Thus, it can be argued that the higher frequency in anticipatory and delayed saccades demonstrated by AD subjects might be a consequence of a failure in working memory (inability to maintain the instructions active while performing the task) and a failure in inhibitory control in saccade performance.

Regarding the latency of both anticipatory and delayed saccades, results differed in both tasks. In the PS task, AD subjects also presented increased latencies in all subtasks compared to CTRL. However, MCI group performed differently in these two eye movement variables. Regarding the latency of anticipatory saccades, they had increased latencies similar to AD ($p>.05$), however they evidenced decreased latencies in delayed saccades, similar to CTRL individuals ($p>.05$). It is important to mention that a ‘Sub-task interaction’ was observed and latency times only differed between ‘Simple’ and ‘Overlap’ conditions in both variables, which is in line with the literature (Yang et al., 2011), given the demanding cognitive nature of the ‘Overlap’ sub-task. Nonetheless, this result goes against previous findings (Peltsch et al., 2014), where MCI showed a similar latency of anticipatory saccades to CTRL. However, their sample was composed by single domain aMCI subjects, whereas the present sample includes mostly multiple domains MCI, which explain their similar performance to AD

subjects. It can also be argued that when cognition is more affected in MCI, the increase in anticipatory latencies might reflect an impairment in inhibitory processes related to saccate initiation. Concerning latency of delayed saccades, the relatively preserved processing speed of information in MCI might prevent them for making an excessive number of delayed saccades, hence the similar performance to CTRL.

When looking at the anticipatory latencies in the AS task, results have to be interpreted more cautiously. MCI subjects showed the fastest latency of all groups and they were significantly different than CTRL and AD, who presented similar latencies. This finding was also found by other authors (Peltsch et al., 2014) and it can be explained by the fact that intra-subject variability of scores was too high across groups, which prevents a further discussion on this variable. Delayed latencies in AS were similarly impaired to the PS task among all groups, given that no effect for 'task' was found. A wide latency distribution in oculomotor measures in neurodegenerative conditions has been previously described in the literature (Kapoula et al., 2010), but no consensus about its explanation has yet been achieved. Future studies should consider monitoring anticipatory and delayed latency distributions in MCI and AD, as they can be a good index of inhibition and executive attention decline in the progression to AD.

In the present study, the 'Gap effect' was calculated as a measure of automatic saccade control. This is explained by the inhibition of the fixation cells and disinhibition of movement cells in the SC, responsible for saccade generation. This mechanism is modulated by selective attention (Pratt et al., 2006). As expected, AS task exhibited a larger 'Gap effect' than PS task, however no differences were found among the three groups. However, CTRL and MCI groups exhibited a larger 'Gap effect' in the AS task, suggesting that AD do not benefit as much from the time gap. The 'Gap effect' seem

to be relatively preserved in healthy ageing (Noiret et al., 2016; Peltsch et al., 2011) and the same pattern is observed in MCI and AD, as shown by previous findings (Peltsch et al., 2014; Crawford et al., 2013; Abel et al., 2002). This indicates that the ability to benefit from externally controlled stimulus disengagement is preserved from healthy ageing to neurodegenerative processes observed MCI and AD.

Conversely, the 'AS cost' is a measure of voluntary saccadic control, determining the additional time needed for AS processes to occur - inhibition of automatic saccade and the voluntary initiation of the AS. Although 'AS cost' seem to be affected by ageing (Peltsch et al. 2011), previous findings reported a significantly increased 'AS cost' in MCI and AD in comparison to healthy ageing (Peltsch et al., 2014). The present results corroborate the literature by showing an increased 'AS cost' in AD subjects compared to CTRL subjects, with MCI demonstrating an intermediate impairment. This result possibly reflects additional difficulties showed by both patient groups with the high-demanding cognitive processes involved in AS task, such as goal maintenance and inhibitory control of reflexive saccades.

Both 'Gap Effect' and 'AS cost' seem to corroborate the differences observed in the different AS oculomotor metrics analysed in this study, confirming that high-order executive cognitive domains seem to be more affected in neurodegenerative conditions than the more automatic visual processing. It is important to understand which cognitive processes are behind these oculomotor patterns and what do they reveal about the executive decline observed in each group. Next, the association between different measures of executive decline and the oculomotor metrics studied will be reviewed.

7.3. Association between neuropsychological and eye movement measures

One of the goals of the present study was to identify an oculomotor profile associated to executive function impairments in each group. Pearson's correlations and multiple regression analyses were conducted to determine the relationship between eye movement variables in both tasks and neuropsychological measures. The relationship between these measures in the PS task will be briefly discussed as they reflect more automatic processes of saccadic control and a more detailed reflexion will be conducted on the AS task, given its executive nature.

The correlations between oculomotor and neuropsychological measures in the PS task are modest, which seems to corroborate the lack of differences between groups in this task. It is worth mentioning that most studies using saccadic eye movements and specific neuropsychological measures have failed to analyse correlations in the PS tasks, restricting their analyses to the AS task. Despite this fact, the present results are consistent with previous studies (Noiret et al., 2018; Peltsch et al., 2014), where the same lack of correlations in the PS task was observed. In fact, PS task involves more automatic, stimulus-driven saccades (Hallett, 1978), with a more limited cognitive processing. Nevertheless, the present study found specific correlations between anticipatory saccades and most of the neuropsychological measures, such as memory, attention, inhibitory control, working memory and verbal fluency. Another interesting finding was the correlation of the working memory measure (BDS) with almost all of the eye movement metrics in this task. The same correlations were observed in the AS task, given that no 'Task effect' was found for the 'anticipatory saccades' variable. Nonetheless, the correlations found for this variable in the PS task, corroborate the fact that anticipatory saccades are not purely an automatic visual processing error but are rather influenced by top-down cognitive

control, namely inhibitory control, self-monitoring and goal maintenance. A more detailed discussion about these correlations will be presented next in this chapter, when addressing correlations in the AS task.

The association between AS task metrics and neuropsychological measures will be the focus of this discussion, due to the executive nature of this task. Overall, AS oculomotor measures correlated significantly with measures of executive function (after controlling for age and education), similarly to previous reports in the literature (Noiret et al., 2018; Holden et al., 2018; Peltsch et al., 2014; Boxer et al., 2006). Specifically, eye movement measures showed moderate correlations with measures of attention, inhibitory control, working memory and verbal fluency. This confirm the validity of the AS task as a measure of executive function.

The percentage of correct saccades and errors presented correlations with several neuropsychological measures, namely with memory scores, BDS, FAS, Stroop sub-tests and TMT-A. These associations reflect the high cognitive demand of the AS task, i.e., the inhibition of an automatic reflexive saccade towards the target (erroneous PS) and the initiation of a voluntary saccade opposite to the target. Several authors have mentioned the utility of AS errors as a sensitive measure of executive function decline, namely inhibitory deficits (Peltsch et al., 2014; Heuer et al., 2013; Mirsky et al. 2011), which was corroborated by this study. However, the present results added that correct saccades are also a good measure of executive functioning, given their association with several cognitive measures. This association was also reported by Garbutt and colleagues (2008), who observed that the percentage of correct AS was the best measure distinguishing AD subjects from other groups.

As discussed previously in this chapter, one of the main oculomotor impairments in AD was the low percentage of correct saccades, with a high proportion

of errors and corrected saccades compared to CTRLs. The MCI group had an intermediate percentage of correct saccades, a lower percentage of errors than AD (similar to CTRL's performance) and they corrected more errors than AD and less than CTRL group. Given the decreased percentage of correct saccades and the elevated number of errors showed by AD subjects in this task, it can be argued that they are markedly impaired in inhibitory control necessary to avoid an automatic saccade. All the more, Crawford and colleagues (2013), observed that the percentage of errors was unaffected in patients with Parkinson's disease or in the healthy control group. These results clearly demonstrated that these errors are not a general characteristic of a neurodegenerative conditions or healthy aging, but can be related specific to AD.

However, inhibitory control is not the only affected cognitive process when making an AS. Simultaneously, they present deficits in working memory, attention, self-monitoring and cognitive flexibility needed when programing and initiating a voluntary anti-saccade. Conscious awareness of the error, continuous recall of the task instruction and the initiation of a volitional saccade are involved when performing an AS. The significant increased number of corrected saccades in the AD group compared to CTRL group, demonstrates a clear impairment of these functions, as demonstrated by other studies before (Crawford et al., 2005). The percentage of corrected saccades correlated positively with the Stroop test (although not with Stroop - errors) and negatively with BDS (measure of working memory) and FAS (measure of verbal fluency, self-monitoring, inhibition, cognitive flexibility, processing speed). The ability to maintain the task goal and rapidly correct a saccade triggered toward the target needed when executing a corrective saccade, requires an efficient executive-attention system and working memory. It is worthwhile to mention that the number of corrected saccades was not significantly associated to any measure of

attention (TMT-A/B, FDS), as opposed to what was observed in the number of errors, where a positive association was found. It can then be inferred that the attentional process needed to initiate a volitional (corrective) saccade has to be relatively preserved when correcting erroneous saccades.

This might suggest that AS errors (that were not corrected) observed in our sample may not reflect exclusively deficits in inhibiting an erroneous automatic saccade. The fact that even AD subjects showed a higher percentage of corrected errors than uncorrected errors is a clear evidence that, despite their working memory is partially affected, they tend to not forget the context of the task. It appears to be the unreliable self-monitoring of the error and correction function that is a major problem for the AD group, as reported by a previous study (Crawford et al., 2013). Then, it can be assumed these patients present an executive-attention control deficit when dealing with the competition between inhibiting a saccade towards the target location and correctly recalling the intended location of the eye movements (opposite hemifield). This hypothesis would also explain what has been reported by previous studies that found correlations between AS errors and measures of inhibitory control (Petlsch et al., 2014; Heuer et al., 2013) together with working memory (Heuer et al., 2013), attention (Holden et al., 2018; Peltsch et al., 2014; Heuer et al., 2013) and verbal fluency (Holden et al., 2018). Interestingly, in a study with healthy controls, Mirsky and collaborators (2011) found that the percentage of correct AS responses was mostly explained by specific measures of attentional set-shifting and generation /initiation of actions. When looking at percentage of errors and corrected saccades in MCI subjects, they present a similar number of errors to healthy controls and an intermediate percentage of corrected saccades between the other groups. This might suggest that, despite being partially affected in their executive attention (observed by

the intermediate number of corrected saccades between CTRL and AD), MCI individuals are relatively preserved in their working memory processes and inhibitory control (as shown by the low percentage of errors).

Measures of saccade latency showed few correlations with neuropsychological measures, which can be partially explained by the high variability of these data. However, latency of correct saccades, a measure of voluntary saccadic control, presented significant correlations with measures of attention (TMT-A, FDS), information processing speed and inhibitory control (both measured by Stroop sub-tests). The present study revealed that AD subjects were significantly slower at initiating a saccade in the opposite direction of the target. This finding is consistent with previous results in the literature (Noiret et al., 2018; Peltsch et al., 2014) that associated AS correct latency to measures of attention. Thus, it can be suggested that voluntary saccade initiation depends on attentional processes, an ability that is known from early stages in AD (Johns et al., 2012). Brain areas such as FEFs, DLPFC and SEFs are involved in voluntary saccade initiation and they are also known to be affected in AD pathology, as previously discussed in this study. In this study, latency suggests that MCI and CTRL subjects were faster than AD subjects in shifting their attention and making a decision when initiating a correct AS. Although very few studies addressed saccadic latencies in MCI, this finding is also consistent with the literature (Peltsch et al., 2014; Yang et al., 2013). Previous studies proved that saccadic latencies decline with healthy ageing due to a general decline in processing speed and not due to specific executive deficits (Noiret et al., 2016). Thus, it can be concluded that MCI subjects are preserved in their ability to initiate voluntary saccades as much as healthy controls do.

Latencies of corrected saccades were positively associated with measures of information processing speed and inhibitory control in the overlap condition (Stroop sub-tests) and negatively associated with verbal fluency (FAS). These results are in contrast to previous findings (Peltsch et al., 2014), which failed to find any correlation between these measures. These contrasting results could be due to the fact that the authors only used the frequency of errors in Stroop test as a measure of inhibitory control, whereas in the present study all sub-scores from Stroop were used (including time-dependent measures - Stroop 'W' and Stroop 'C'). AD subjects showed significantly greater corrective latencies compared to CTRL subjects, as reported by other authors (Noiret et al., 2018). As discussed previously in this chapter, MCI subjects made less corrected saccades than AD (due to a lower percentage of errors), however they exhibited a greater latency when correcting an error, similar to AD group. It can be argued that both patient groups demonstrate a significant deficit in their reaction times when inhibiting an automatic saccade as well as in self-monitoring and in cognitive flexibility. These two latter cognitive domains are needed to recognise an error and to redirect the attention to initiate a volitional (corrective) saccade. This finding is consistent with the correlation found between the latency of corrected saccades and Stroop and FAS test. It is possible to speculate that, given the partial preservation of goal maintenance capacities in MCI subjects, they are able to maintain a lower error rate similarly to controls but only when they increase their response times. As suggested by Braver and colleagues (2008), inhibition is then the result of a combined action of reactive control (detection and suppression of interference) and proactive control (the use of context to constantly recall the relevant goal).

No significant correlations were found between the latency of errors in AS task and neuropsychological measures, apart from a positive association with TMT-B, a

measure of selective attention, which was also found in previous works (Noiret et al., 2018). AD subjects evidenced an increased latency when committing errors compared to healthy controls. This can be due to an impairment of selective attention when triggering an erroneous automatic saccade towards the target. Furthermore, the fact that error latency didn't show any group differences in the PS task corroborates that AS error latency in AD are not simply due to a decline in processing information speed.

Anticipatory and Delayed saccades exhibited many correlations with standard neuropsychological scores in the present study. This suggests that a high order cognitive control might have some influence over saccade parameters that are usually considered to be automatic. Anticipatory saccades correlated positively with measures of attention (TMT- A/B), information processing speed and inhibition (Stroop ‘’W, ‘C’, ‘WC’), working memory (BDS) and fluency (FAS). This association was corroborated by the multiple regression analysis performed in this study, where ‘anticipatory saccades’ was one of the oculomotor metrics that seemed to explain the performance of almost all the cognitive measures. Similar correlations were also found by other authors (Noiret et al., 2018), especially with attention / executive function measures. AD group had an increased number of anticipatory saccades compared to CTRL, even in the ‘Gap’ sub-task where usually the disengagement of visual attention increases the number of anticipations for all groups. Some authors attribute this failure of active fixation in the presence of the upcoming need to make a saccade to degeneration in the posterior parietal cortex or *substantia nigra pars reticulata* (SNpr; Csibra et al. 1997). The correlation found in the present study between anticipatory saccades and measures of information processing speed and inhibitory control (Stroop sub-tasks) seem to corroborate this finding. Hotson and Steinke (1988) argued that anticipatory saccades reflect an inability to remember task instructions, which is supported by our

data showing a correlation between working memory measures and this eye movement metric. Other authors have also argued that top-down cognitive processes such as working memory are involved in target anticipation (Hutton et al., 2001). MCI patients showed more anticipatory saccades than CTRL but less than AD patients, possibly due to an intermediary deficit in working memory and executive-functioning, as previously seen in this study.

Delayed saccades also exhibited correlations with measures of information processing speed, working memory and fluency. So far the literature has been very scarce regarding cognitive control related to delayed saccade, and no study could be found addressing this topic. However, it is fair to say that the increase frequency of delayed saccades seen in AD patients compared to healthy controls could be related to an impairment of information processing speed but also due to a failure in self-monitoring and in the maintenance of goal activation during saccadic performance. No correlations were found between anticipatory and delayed latency and any neuropsychological measure, as observed in previous studies (Noiret et al., 2018; Abel et al., 2002), which can be partially explained by the high variability of these scores.

An interesting finding was the correlations found between specific memory scores and several eye movement variables, as previously found by other authors (Holden et al., 2018; Noiret et al. 2018; Boxer et al., 2006). As discussed previously in this chapter, attentional and executive processes are required during the encoding and consolidation stages of memory processing (implementation of semantic strategies to facilitate encoding) and they significantly affect short and long term memory scores in MCI and AD groups. Thus, it can be suggested that memory recall

performance in these groups could be partially explained by executive function impairments.

Although multiple regression analyses exhibited modest results, they reinforce the association found in the correlations between oculomotor and neuropsychological measures. Overall, all oculomotor metrics accounted in some degree for the score variance of the different executive function domains studied. An interesting finding was the fact that 'anticipatory saccades' was the oculomotor variable that influenced more executive function sub-domains, as opposed to previous results, where the frequency of errors best distinguished between groups or best explained working memory performance among specific clinical populations (Crawford et al., 2013). However, it is worth mentioning that most studies discard anticipatory saccades from the analyses. The present results seem to corroborate the idea that this type of saccade cannot be considered merely an 'automatic' error but rather reflect possible executive impairments in inhibition and executive attention control. While other studies focused on finding one single oculomotor metric that best explains the variance of each cognitive domain (Crawford et al., 2013; Shafiq-Antonacci et al., 2003), the present study highlights the importance of finding a more robust group of oculomotor metrics that can possibly bring a more comprehensive description of executive functioning performance in clinical populations, such as MCI and AD.

Previous studies have confirmed that the dorsolateral prefrontal cortex, frontal eye fields, supplemental eye fields and inferior frontal junction areas are involved in the AS task performance, specifically in saccade inhibition and voluntary saccade initiation (Leigh & Kennard, 2004; Ettinger et al., 2005). Also, it is known that ageing affects voluntary saccades, 'AS cost' and the number of errors and that the previously described brain structures responsible for AS generation are less resilient to the

ageing process (Peltsch et al., 2011). Furthermore, Mirsky and colleagues (2011) found an association between correct AS and structural alterations within the same frontal lobe inhibitory control network nodes in healthy controls and in patients with dementia. In addition, they observed that differences in the severity of AS impairment reflected different degrees of impairment in inhibitory control brain network. Thus, it can be argued that these structural deficits found in elderly healthy controls can be related to degenerative processes due to ageing but they can also reflect an incipient neurodegenerative condition, such as AD. Similarly, recent findings showed a decreased activation in frontal eye fields in aMCI patients compared to healthy elderly controls during the performance of an AS task (Alichniewicz et al., 2013). This seems to corroborate the idea that the frontoparietal oculomotor network may contribute to predict the conversion to AD. Altogether, these findings prove that the AS task is sensitive to executive functioning decline related to MCI and AD conditions.

Overall, the AS task proved to be a useful measure of executive function from healthy ageing to Alzheimer's disease. Although it is influenced by ageing, it accurately measures dysexecutive impairments in AD and prior to the disease, in MCI condition.

One of the goals of this study was to determine an oculomotor profile associated with executive functioning impairments in healthy controls, subjects with MCI and subjects with AD. In fact, it was observed that all three groups exhibited a different oculomotor profile, reflecting different levels of executive impairment. AD subjects exhibited a greater decline than CTRL subjects in inhibitory control, working memory, self-monitoring and executive-attention control, as indicated by an increased number or errors, less correct saccades, an increase in corrected saccades, anticipatory saccades and a greater 'AS cost'. MCI subjects exhibited an intermediate level of impairment in their executive functioning, compared to the other groups.

Interestingly, they exhibited a lower error rate due to the partial preservation of working memory and inhibition. However, they are only able to maintain a lower error rate with an increase in their response times, as shown by the increased latencies of their corrective saccades, similar to AD patients. This indicates an impairment of executive attentional control when dealing with highly demanding cognitive tasks and may constitute a good predictor of the progression to AD.

7.4. Diagnostic value of eye movement variables

One of the specific goals of the present study was to determine if the oculomotor measures associated to executive function impairments could distinguish between groups. For that, a logistic regression was performed comparing the groups with each other (CTRL vs. MCI, CTRL vs. AD, MCI vs. AD).

ROC curve analysis showed that oculomotor variables distinguished between healthy controls and AD patients with a good accuracy (79.3%). The same pattern was observed when separating healthy controls from MCI individuals, with oculomotor variables showing a moderate accuracy (66.6%) distinguishing between the groups. This distinction is of great importance in clinical practice, as it allows not only an early detection of individuals at risk of developing AD but it also helps monitoring progression to the disease. Due to the existence of healthy control individuals that might be at preclinical stage of neurodegenerative diseases, the likelihood of behavioral markers to classify subjects in mild stage of the disease with perfect accuracy decreases. MCI and AD patients were separated with an accuracy of around 78%. When looking at the set of oculomotor variables that separated between groups, they all corroborate the differences between groups observed for each single oculomotor parameter discussed previously in this chapter. These results are in

accordance with previous studies in the literature, where eye movement variables demonstrated a good performance separating between AD patients and other clinical groups / healthy controls (Chehrehnegar et al., 2019; Shakespeare et al., 2015; Garbutt et al., 2008).

Whilst it cannot be proposed that eye movement analysis should be used in isolation as a diagnostic test, the results suggest that oculomotor paradigms are also informative at the individual level. Future studies using machine learning methods and eye tracking paradigms may improve considerably classification accuracy of these populations, as it has been proved before (Pavisic et al., 2017; Lagun et al., 2011). In a previous research study conducted by LIM-27 using eye tracking metrics to study visual search efficiency in MCI / AD (Appendix B), it was possible not only to distinguish between healthy controls and AD subjects, but also to identify MCI subjects who had an oculomotor performance similar to either healthy elders or AD patients.

To the best of our knowledge, only two studies (Peltsch et al., 2014; Heuer et al., 2013) have previously compared eye movement profiles from MCI and AD groups. Most studies limited their analyses to subjects either with MCI or AD and they mainly used the PS task as a paradigm. Moreover, the eye movement metrics analysed in these studies were not as extensive as the one used in the present study and only one of these studies used a more comprehensive neuropsychological battery of tests (Peltsch et al., 2014). The present study added to the described research because it was one of the very few that compared MCI and AD oculomotor profiles using both PS and AS paradigms, with a comprehensive assessment of executive functions in these groups.

It must be acknowledged that the present study may have some potential limitations. The MCI sample included different sub-types (amnestic, non-amnestic),

but mostly multiple-domain MCI subjects. The significance of the present results highlights that the analysis of eye movement behaviour is sensitive to particular profiles of MCI at a higher risk of converting to dementia. Nonetheless, a separation of MCI samples according to their sub-types may help to examine the following concepts further - to what extent can an amnestic profile of MCI exhibit early dysexecutive impairments? Could eye movement measures be sensitive for identifying executive dysfunction in parallel to deficits in memory prior to the onset of AD?

Despite the fact that some differences were found regarding saccadic latencies and anticipatory saccades in the different sub-tasks (simple, gap, overlap), this study used a limited number of trials per sub-task in both PS / AS paradigms. This prevented us from finding further 'sub-task' effects among groups. It would be beneficial in future studies to add more trials to each sub-task in order to draw further conclusions about abnormalities in eye movements in MCI / AD conditions.

Another addition that would result in clearer diagnostic results would be to combine mixed paradigms, such as eye movement tasks with fMRI. This would provide insightful information on how functional brain changes relate to eye movement patterns in MCI and AD patients compared with healthy controls.

Although clear relationships between eye movements and specific neuropsychological measures of executive functions were found, it would be useful to carry out correlations with dementia rating tests, to better describe the potential relationships between eye movement metrics and objective levels of dementia in AD.

Finally, studies that follow MCI patients longitudinally to determine precise rates of conversion are imperative in order to improve diagnostic accuracy in AD.

8. CONCLUSION

8. CONCLUSION

1. Elderly controls, MCI and AD exhibited a different performance in all cognitive domains assessed, as shown by neuropsychological measures. Globally, AD showed decline in executive functioning compared to elderly controls, with MCI showing an intermediate decline between the other groups.

2. The AS task is a useful tool in assessing executive dysfunction in healthy ageing and in neurodegenerative conditions, such as MCI and AD.

3. Different oculomotor patterns were found in elderly controls, subjects with MCI and in subjects with AD. Elderly controls exhibited a mild impairment in their oculomotor patterns with a worse performance in the AS task, compared to the PS task. AD subjects showed a lower number of correct saccades, a higher number or errors, corrected saccades, anticipatory saccades and a greater 'AS cost' than elderly controls. MCI made a similar number of errors to CTRL, with an intermediate impairment in the number of correct, corrected, anticipatory, 'AS cost'. Interestingly they showed a similar latency of corrected saccades to AD subjects.

4. The different oculomotor patterns found in elderly controls, subjects with MCI and in subjects with AD reflect different levels of executive impairment. In elderly controls the worse performance in AS indicates a mild executive decline in more demanding cognitive tasks, specifically in processing speed and attention. AD subjects exhibited a greater decline than CTRL subjects in processing speed, inhibitory control, working memory, self-monitoring and executive-attention control. MCI subjects exhibited an

intermediate level of executive functions impairment compared to the other groups. They increase their reaction times in order to avoid errors, as shown by the increased latency of corrected saccades. This suggests an impairment of executive attentional control when dealing with highly demanding cognitive tasks and may constitute a good predictor of the progression to AD.

5. Oculomotor measures were able to separate between elderly controls, subjects with MCI and subjects with AD with a good accuracy.

9. REFERENCES

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APPENDICES

A. Written informed consent

HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO - HCFMUSP

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

DADOS SOBRE A PESQUISA

1. TÍTULO DO PROTOCOLO DE PESQUISA: O Funcionamento Executivo como indicador de conversão para a Doença de Alzheimer: contributo da análise do movimento ocular.

PESQUISADOR PRINCIPAL: Dr. Orestes Vicente Forlenza
DEPARTAMENTO / INSTITUTO: Departamento e Instituto de Psiquiatria da Faculdade de Medicina da Universidade de São Paulo – Laboratório de Neurociência (LIM-27).

ENDEREÇO: R. Dr. Ovídio Pires de Campos, 785. 3o Andar – LIM 27 – Tel: 26616132. Caixa Postal 3671. CEP 01060-970. São Paulo – SP

CONVITE À PARTICIPAÇÃO

Convidamos o(a) Sr.(a) para participar desta pesquisa com o título "O Funcionamento Executivo como indicador de conversão para a Doença de Alzheimer: contributo da análise do movimento ocular", em que faremos (falar movimento ocular) um teste em um monitor de um computador onde ficará registrado o seu movimento ocular.

1. Justificativa e objetivos do estudo: As alterações cognitivas prévias aos sintomas clínicos da doença de Alzheimer (DA) são alvo de várias pesquisas atuais, sendo que as alterações "clássicas" de memória episódica e semântica são colocadas em causa como sendo os únicos sinais precoces da doença. Os dados mais recentes mostram que alterações do funcionamento executivo estão presentes antes do estabelecimento da DA, nomeadamente no Comprometimento Cognitivo Ligeiro (CCL), porém, ainda é desconhecida a afeção nos diferentes sub-domínios executivos. A utilização de tecnologia de rastreamento ocular tem-se revelado um método não-invasivo e com uma boa relação custo-eficácia na pesquisa de marcadores da DA, nomeadamente de padrões comportamentais como as alterações executivas em fases pré-demenciais. Por isso, o presente estudo pretende investigar as alterações do funcionamento executivo preditivas da DA em sujeitos com CCL, através do seu perfil oculomotor.

2. Descrição dos procedimentos que serão realizados e métodos que serão utilizados: Se você decidir participar, passará pelo protocolo de pesquisa do LIM-27 que consiste em avaliação clínica, assim como pelas avaliações neuropsicológica e funcional, que consistem na aplicação de testes padronizados para comparação de seu desempenho com outros indivíduos da mesma idade e escolaridade. Realizará também alguns testes em um monitor de um computador, onde será registrado o seu movimento ocular.

Os dados serão avaliados em corte transversal, correlacionando-se os mesmos com variáveis clínicas, neuropsicológicas e funcionais.

3. Desconfortos e riscos esperados: Os procedimentos a serem realizados não oferecem qualquer tipo de risco ou desconforto para os voluntários. Toda a testagem leva aproximadamente 2,5 horas e envolve apenas a observação de imagens em um monitor de computador, ou a execução de tarefas envolvendo lápis e papel. A equipe de profissionais, que inclui neuropsicólogos e terapeutas ocupacionais do LIM-27, tem grande experiência na condução de avaliações semelhantes a essa, além de receberem treinamento específico para a realização dos procedimentos previstos neste projeto, visando garantindo conforto e agilidade na realização dos procedimentos.

4. Benefícios que poderão ser obtidos – Não há benefício garantido para o participante; trata-se de um estudo experimental para testar a capacidade do equipamento e da metodologia de análise do movimento ocular de detectar alterações precoces em pacientes com déficit cognitivo possivelmente relacionado à Doença Alzheimer.

5. Acesso, a qualquer tempo, às informações sobre procedimentos, riscos e benefícios relacionados à pesquisa, inclusive para esclarecimento de eventuais dúvidas. Você pode perguntar sobre qualquer dúvida que tenha agora ou em qualquer momento deste estudo e poderá ter acesso às informações sobre os procedimentos, riscos e benefícios relacionados à pesquisa. Os voluntários poderão beneficiar do seguimento clínico, das avaliações cuidadosas de várias funções cognitivas.

6. Liberdade de retirar seu consentimento a qualquer momento e de deixar de participar do estudo, sem que isto traga prejuízo à continuidade da assistência. Você pode recusar-se a participar deste estudo ou pode retirar o seu consentimento e descontinuar sua participação a qualquer momento, sem que haja qualquer punição ou perda de benefício aos quais tem direito.

7. Recebimento de via do termo de consentimento livre e esclarecido. Após a assinatura das duas vias do termo de consentimento livre e esclarecido, pelo responsável pelo projeto e pelo participante (ou seu representante legal), uma das vias será entregue ao participante, sendo que a outra ficará com a equipe de pesquisa.

8. Salvaguarda da confidencialidade, sigilo e privacidade. Os registros que identificarem você pelo seu nome serão mantidos confidenciais e nenhum documento que o identifique sairá do Hospital. Se os resultados forem publicados, sua identidade permanecerá absolutamente confidencial.

9. Disponibilidade de assistência no HCFMUSP, por eventuais danos à saúde, decorrentes da pesquisa. Como esclarecido acima, os riscos relacionados a esta pesquisa são mínimos. Todavia, garantimos a disponibilidade de assistência no HC/FMUSP para cobrir qualquer dano a sua saúde, por menor que seja, diretamente decorrente desta pesquisa.

10. Viabilidade de indenização por eventuais danos à saúde decorrentes da pesquisa. Vide item 8.

Em qualquer etapa do estudo, você terá acesso aos profissionais responsáveis pela pesquisa para esclarecimento de dúvidas. O principal investigador é o Dr. Orestes Vicente Forlenza que pode ser

encontrado no endereço R. Dr. Ovídio Pires de Campos, 785. 3o Andar – LIM 27 – Tel: 2661 6132. Caixa Postal 3671. CEP 01060-970. São Paulo – SP, e-mail: pesquisamemorialim27@gmail.com. Se você tiver

alguma consideração ou dúvida sobre a ética da pesquisa, entre em contato com o Comitê de Ética em Pesquisa (CEP) – Rua Ovídio Pires de Campos, 225 – 5o andar – tel: (11) 2661-7585, (11) 2661-1548, (11) 2661-1549; e-mail: cappesq.adm@hc.fm.usp.br

Fui suficientemente informado a respeito do estudo “O Funcionamento Executivo como indicador de conversão para a Doença de Alzheimer: contributo da análise do movimento ocular”. Eu discuti as informações acima com o Pesquisador Responsável (Dr. Orestes Vicente Forlenza) ou pessoa (s) por ele delegada (s) (Marta Luísa Gonçalves de Freitas Pereira; Marina von Zuben de Arruda Camargo; Ariella Fornachari Belan) sobre a minha decisão em participar nesse estudo. Ficaram claros para mim os objetivos, os procedimentos, os potenciais desconfortos e riscos e as garantias. Concordo voluntariamente em participar deste estudo, assino este termo de consentimento e recebo um via rubricada pelo pesquisador.

Assinatura do participante /representante legal Data / /

Assinatura do responsável pelo estudo Data / /

DADOS DE IDENTIFICAÇÃO (OU ETIQUETA INSTITUCIONAL DE IDENTIFICAÇÃO) DO PARTICIPANTE DA PESQUISA OU RESPONSÁVEL LEGAL

1.NOME: .....

DOCUMENTO DE IDENTIDADE No : ..... SEXO : .M ☐ F ☐
DATA NASCIMENTO: ...../...../.....
ENDEREÇO ..... No ..... APTO: BAIRRO:
..... CIDADE:.....
CEP:..... TELEFONE: DDD (.....) .....

2.RESPONSÁVEL LEGAL .....
NATUREZA (grau de parentesco, tutor, curador etc.) .....

DOCUMENTO DE IDENTIDADE :..... SEXO: M ☐ F ☐

DATA NASCIMENTO.: ...../...../.....
ENDEREÇO: ..... No ..... APTO: .....
BAIRRO: ..... CIDADE:..... CEP:
..... TELEFONE: DDD (.....).....

B. Article submitted

"Visual search efficiency in mild cognitive impairment and Alzheimer's disease: an eye movement study."

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Abstract:

Background: Visual search abilities are essential to everyday life activities and are known to be affected in Alzheimer's disease (AD). However, little is known about visual search efficiency in mild cognitive impairment (MCI), a transitive state between normal aging and dementia. Eye movement studies and machine learning methods have been recently used to detect oculomotor impairments in individuals with dementia. Objective: The aim of the present study is to investigate the association between eye movement metrics and visual search impairment in MCI and AD. Methods: 127 participants were tested: 43 healthy controls, 51 with MCI and 33 with AD. They completed an eyetracking visual search task where they had to find a previously seen target stimulus among distractors. Results: Both patient groups made more fixations on the screen more often when searching for a target and spent more time doing it than controls. MCI and AD fixated the distractors more often and for a longer period of time than the target. Healthy controls were quicker and made less fixations when screening stimuli for the first time. Machine-learning methods were able to distinguish between controls and AD subjects and to identify MCI subjects with a similar oculomotor profile to AD with a good accuracy. Conclusion: Results showed that eye movement metrics are useful for identifying visual search impairments in MCI and AD, with possible implications in the early identification of individuals with high-risk of developing AD.

Keywords: Alzheimer's disease; Mild Cognitive Impairment; Eyetracking; eye movements; machine learning; visual search; visual attention; visual impairments.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most prevalent type of dementia in older adults. It is characterized by early impairment of episodic and semantic memory, and recent findings have shown early deficits in other cognitive domains such as executive functions, attention and visuospatial abilities. Visual processing skills have also been tentatively studied, but so far yielding inconsistent results. Visual deficits may be related to both ventral and dorsal pathways.[1] The distribution of neurofibrillary tangles and amyloid deposits in typical AD include the visual system, including the primary visual and association areas. In the dorsal visual pathway, there is a significant loss of long corticocortical projections from early visual areas to medial temporal areas (eg, visual area V5). Greater impairment along the dorsal visual pathway in patients with mild AD patients result in abnormalities in 'lower' levels of visual processing such as color perception, visual acuity, contrast sensitivity and motion perception, and also in 'higher' levels, such as visuospatial reasoning, face and object recognition.[2] Visual search is altered in AD and patients often have an increased difficulty detecting objects either in simple and complex scenes. This difficulty translates into deficits in recognizing salient features, an increased time to detect the target, and difficulty in shifting attention between global and local features.[3,4] Also, AD patients may have a narrower zone of focal attention, with an increased difficulty in shifting between central and peripheral areas of a scene.[5] Neuroimaging studies suggested that AD patients have a reduced parietal activation and increased temporal activation in visuospatial processing, which leads to impairments in 'top-down' control during visual search.[6,7]

Eye tracking technology has been used as a reliable and cost-effective technique to collect visual processing data in clinical populations.[8–11] The minimal verbal or motor requirements subjacent to the method makes it particularly suitable for older participants. Visual impairments have been related to the progression of AD in the literature.[12,13] Hence the analyses of eye

movement has been suggested as a potential and accurate surrogate method of assessing cognitive impairments in the course of AD. These visual deficits translate into impairments in eye movement behaviour, with AD patients showing saccadic intrusions, increased saccade latencies, increased catch-up saccades, deficits in fixation and slow pursuit movement.[14–18] Moreover, oculomotor variables seem to aid in the detection of dorsolateral prefrontal cortex (DLPFC) degeneration in AD. These patients seem to make more incorrect saccades towards the target in an antisaccade task[19,20], with fewer corrective saccades after the error, and increased latencies when performing this task. These variables seem to correlate with cognitive measures of inhibition, attention and working memory.[21,22]

Eye movement metrics may also be useful in the assessment of visual deficits in AD patients when performing basic and high-order visual perceptive tasks. Mosimann and colleagues[23] analysed visual exploration in AD patients during a clock-reading task and collected eye movement data while the patients performed the task. They observed that these subjects had different patterns of visual exploration, with a less focused exploration with fewer fixations inside the regions of interest (ROI), longer fixations in different areas and smaller saccade amplitudes. Healthy controls tend to focus their attention in more central areas of visual scenes, whereas AD patients seem to spread their gaze mostly in peripheral areas of these fields, exhibiting more fixations with an increased duration in these peripheral ROI. This could be explained by an impairment in disengaging their attention from peripheral targets.[24]

Recently, the literature has been pointing out early visual impairments in mild cognitive impairment (MCI), an intermediate condition between normal ageing and dementia, with an increased risk of progression to AD.[25] Some studies have found memory impairments in MCI using eye movement metrics with novelty preference tasks,[26,27] where the number of fixations and fixation duration was related to short-term memory impairments and damages to the hippocampus in MCI subjects. Also, more recently some authors have used eye movement

representation features and machine learning methods to improve diagnostic accuracy by distinguishing MCI subject from healthy controls with high accuracy rates.[28,29]

Attention is also early affected in MCI. In a study comparing different types of visual attention, MCI patients evidenced greater deficits in divided attention as compared to cognitively unimpaired elders.[30] In addition, antisaccade oculomotor measures were found to identify subtle executive changes in MCI similar to those found in subjects with AD, in particular selective attention and inhibitory control.[31–33] In these studies, both patient groups (MCI and AD) displayed a similar oculomotor pattern with slower correct saccades and a higher number of antisaccade errors. These impairments had strong correlations with neuropsychological measures of selective attention and executive function. Altogether, these findings reflect decreased activation in frontal eye fields in MCI patients, and bring new insights into early manifestations of AD pathology.[14,33,34]

On the other hand, while visuospatial deficits have been associated with cognitive decline typical of AD, is it still less clear to which extent visuospatial abilities are affected in MCI, specifically if there is a particular pattern of visual exploration associated to this condition. Recent findings suggest that top-down attentional control is affected in MCI, namely a reduction of attention shifting in visual search efficiency.[35,36] However, little is known about the oculomotor metrics that best describe this decline in MCI patients.

A growing number of studies indicate that machine learning represents a valuable method to integrate large-scale datasets in the diagnosis of complex clinical conditions such as AD. Thus, algorithm-based approaches are likely to bring more accuracy by using multiple features of different subjects to classify them into different clinical groups.[28,37,38] The incorporation of analytical methods using machine learning in *eyetracking* studies may contribute not only to the identification of early cognitive changes in oligo-symptomatic individuals that might go

unnoticed by more traditional methods of cognitive assessment, but also to monitor disease progression over time with high sensitivity to detect subtle decline.

The aim of the present study was to investigate the association between eye movement metrics and visual search impairment in a sample of older adults with MCI and AD, as compared to cognitively unimpaired elders (healthy controls). We hypothesize that subjects with mild or severe impairments will display abnormalities in oculomotor behavior, , suggesting that visual search impairment is an early manifestation in the MCI-AD continuum. In addition, using oculomotor metrics, we expect to demonstrate that machine learning classification methods will better discriminate individuals with AD from healthy controls and also help identify the sub-sample of MCI subjects who actually present with a similar oculomotor impairment as observed in AD.

Methods

Participants and setting:

The present study was conducted at a specialized memory clinic located in a tertiary university hospital in São Paulo, Brazil. The study group comprised 127 older adults allocated into three groups according to their global cognitive capacity: controls, if cognitively unimpaired (CTRL group, n=43), mild cognitive impairment (MCI group, n=51) and mild dementia due to Alzheimer's disease (AD group, n=33). Participants were community dwelling outpatients recruited from the hospital catchment area and from psychogeriatric services. A detailed medical, social and family history was obtained for each subject including, when needed, additional information from caregivers or informants. Participants underwent a comprehensive neuropsychological and clinical assessment (clinical, imaging and laboratory data), and the diagnosis was established by a multidisciplinary team (including neurologists, psychiatrists, neuropsychologists and speech therapists). Clinical diagnosis of MCI required evidence of a

decline in baseline function of memory and possibly additional cognitive domains, according to the Mayo Clinic criteria,[39] with the severity of symptoms or consequent functional limitations insufficient to meet diagnostic criteria for dementia, according to the *Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV-R)*. The eligibility criteria for AD patients included diagnosis of possible or probable AD based on the DSM-IV-TR and the *National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association* (NINCDS-ADRDA) criteria.[40] Exclusion criteria were the following: neurological or psychiatric conditions/events, ocular diseases, moderate dementia or other type of dementia, calibration problems or low percentage of eye movement recordings. Demographic information is shown in Table 1. All participants gave their written informed consent prior to inclusion into the study, or in the case of dementia patients, a legally authorized representative. All experimental procedures were approved by the institution's research ethics board, in accordance with the declaration of Helsinki.

Procedures:

Neuropsychological testing:

All participants completed an extended battery of neuropsychological assessment, including: the Brazilian versions of the Rivermead Behavioral Memory Test and the Rey Auditory Verbal Learning Test (memory)[41], Rey-Osterrieth Complex Figure Test[42] - copy and delayed recall tests (visuospatial abilities, visuospatial memory and executive functions), Trail Making Test[43] – versions A and B (visual attention and executive functions - task switching and working memory), Stroop Color-Word Test[44] (inhibition), the Verbal Fluency Test[45] (phonemic fluency) and Wechsler Adult Intelligence Scale-Digit Span test[46] – forward and backward versions (attention span and working memory).

Apparatus and eye movement recording:

Eye movement was registered with a Tobii TX300 eyetracker. The visual angle was calculated using the relative position of the center of the cornea and the pupil with an accuracy of ±0.75°. The system sampling capacity was of 300Hz, processing latency of 1-3.3ms, and the participants were seated at a distance of approximately 65cm from a 23" screen, where the stimuli were presented. An I-VT fixation filter was used with standard settings, as this specific filter provides highly accurate fixation classifications for commonly used eyetracking paradigms. No movement restraint was used and the participants had a range of freedom of head movements of 37x17cm. Each subject was asked to shift their eyes between peripheral and central stimulus locations in order to calibrate the eye tracker, using a 9-point calibration system. Eye movement data were recorded using Tobii Pro Studio® software. Once the calibration was successfully completed, the experimenter started the recording session.

Eye movement paradigm:

The test protocol was designed by the research team using simple drawn images and public domain pictures. The protocol contained 3 clusters of trials. The first was composed of simple geometric forms, the second contained abstract forms and the last cluster included visual scenes. Each trial consisted of a familiarization encoding phase and a test recognition phase. Trials in one cluster contained images with common features. During the task, participants were asked to carefully observe one stimulus on the screen, and to recognize it afterwards among other three distractor images. All participants were asked to explain the task to the experimenter in order to demonstrate their understanding of the tasks requirements before the experiment started. The familiarization phase lasted between 2 and 5 seconds, according to level complexity of the image (clusters 1 and 2 – 2 seconds; cluster 3 – 5 seconds), after which the participant was instructed on how to respond to the following (test) phase. Images presented in the screen in the test phase were numbered 1 to 4. No limit of time was imposed for the

participant to provide his/her answer, meaning that the test phase ended when he/she provided a verbal answer indicating the number of the selected response (image). The correct answer (the same image that had been presented during the familiarization phase) was randomly placed in different areas of the screen (Figure 1). Once this step was accomplished, the command to start the next trial was given by the examiner.

FIGURE 1

Eyetracking variables:

Eye fixation and eye movement data for each participant were extracted and analyzed using Tobii Pro Studio® software. Eye movement measures were analyzed from trial onset to the subject's verbal answer that terminated the trial. Fixation was defined as a point of gaze continually remaining within 1° of visual angle for a period of, at least, 100ms.

The following eye movement characteristics were analyzed in the experiment: (1) time to first fixation (TFF), i.e., time (in milliseconds) taken before the participant fixates gaze on a region of interest (ROI) for the first time; (2) fixations before (FB), i.e., number of fixations before the first fixation on any ROI for the first time; (3) fixation count (FC), i.e., number of fixations made in a specific ROI; (4) duration of fixations (DF), i.e., total duration (in milliseconds) of fixations within an ROI. In the current data analysis, oculomotor parameters were analyzed within two different types of ROI: the ROI where the target is located, and the ROI where the distractors are located.

Statistical analyses:

The present set of data is described as mean and standard deviations for numerical variables and absolute and relative frequencies for factors. Baseline sociodemographic characteristics of groups were compared with one-way ANOVA models, Kruskal-Wallis tests and chi square

tests. Eye movement variables on target and distractor stimuli were compared among groups with generalized linear mixed models (GLMM) for Gaussian (LMM) and Poisson families, to check for any eventual effect of 'Group' (CTRL, MCI, AD), 'Stimulus' (Target, Distractors) and any interaction of 'Group' x 'Stimulus'.

Machine learning models:

The variables considered in this model include the variables previously described and analyzed using statistical software (TFF, FB, FC, DF) and additional filtered variables extracted from Tobii Studio software (first fixation duration - FFD, visit duration - VD, total visit duration - TVD, visit count - VC). Variables were addressed to the regions of interest (ROI) related to target stimuli and distractors. In total, there were 13 trials in the assay. Minimum, maximum, sum, mean and median values were calculated for all variables. In total 1,400 variables were represented in at least 50% of the data in each group, with the data being averaged for all variables and using all screen trials for that. Missing values were not accounted into the final averaged value, resulting in a total of 135 variables. Eight variables were cut out, because variance was equal to zero, i.e. all samples showed the same value, resulting in 127 variables. In order to avoid redundant variables, a Pearson's pairwise correlation was performed and variables with $r \geq 0.98$ were represented by only one randomly chosen variable inside the group. This way 72 variables were represented by 39 variables, resulting in a dataset of 94 non-redundant variables. In order to identify variables to be used in the classification task, three different feature selector algorithms were used: (1) Sequential Feature Selector (SFS), with k spanning 2 to 10, forward and back method with floating and scoring accuracy parameters; (2) Las Vegas Weight (LVW), with iteration 1000, score base 0.8, number of folds 3 and accuracy parameters and (3) Genetic Algorithm (GA) with cv of 5, score accuracy, crossover probability 0.5 and mutation probability of 0.2. Features selected were used in three different machine learning algorithms: Support Vector Machine (SVM), Random Forrest (RF) and Neural

Network (NN). For the SVM algorithm all features were tested with linear, radial basis function (RBF) and polynomial and C and gamma parameters were optimized with GridSearchCV. For the RF model all feature selection model were tested with 300 estimators and balanced weight. For NN only, all variables available or the ones selected by LVM were used, the number of neurons were 55 and hidden layers equaled 2. All algorithms were used from scikit-learn package in python 3.6. Training and test dataset were split into 90% and 10% respectively, and performed 10 fold, which means that the model used was trained, tested and evaluated ten times. The evaluation of each model was performed according to accuracy, precision, recall, F1-score and area under the curve (AUC). Briefly, the accuracy measures the overall rate of true positives and true negatives over all tested classes. Precision represents the rate of true positive overall number of samples classified as positive (true positive + false positive). Recall calculates the rate of true positive over all samples truly positive (true positive + false negative). F1-score is a mean of precision and recall to give a balance addressing both measures. AUC summarized the model used representing a balanced measure of accuracy and specificity. The former calculates the true positive rate and the latter measures the true negative rate. All the calculations were performed 10 times for each model tested.

Results

Sociodemographic analyses were computed on 43 control subjects (33 women), 52 MCI (41 women) and 43 AD (20 women), as presented in Table 1. Mean (M) age [$F(2)= 13.37$; $p=0.001$] and years of education [$F(2)= 22.94$, $p<0.001$] differed between groups, with AD subjects being significantly less educated (AD: M=10.42; SD=5.23) than the other groups (CTRL: M=15.28, SD=2.52; MCI: M=13.10, SD=3.84) and older (M=72.97, SD=6.26) than them (CTRL: M=67.98, SD=7.15; MCI: M=68.62, SD=7.94). Neuropsychological test scores for

each group are also presented in Table 1, indicating, as expected, statistically significant differences across groups.

TABLE 1

The analysis of eye tracking variables (TFF, NF, FC and DF) are schematically presented in Table 2 and Figure 2, and detailed in the following paragraphs.

TABLE 2

FIGURE 2

Time to first fixation (TFF):

In the recognition (test) phase, we found statistically significant differences in mean values for TFF across all groups ($p<0.001$), indicating that, in average, participants in distinct diagnostic groups spent a different amount of time looking at different points on the screen before fixating on a stimulus for the first time, irrespective of this first fixation being on the correct or distractor stimulus. AD subjects had significantly higher mean values for TFF than MCI and CTRLS (AD: M=1.09, SD=1.06; MCI: M=0.97, SD=0.72; CTRL: M=0.86, SD=0.68). A statistically significant difference was also found between the MCI and the other groups, with MCI subjects presenting with intermediate TFF values, i.e., between AD and CTRL subjects. Considering the "stimulus" condition (i.e., target vs. distractors), all 3 groups had a significantly higher TFF on the target than on distractors ($p<0.001$) (Table 2, Figure 2A).

Number of fixations before the first fixation on the target stimulus (FBT):

ANOVA revealed that both patient groups (MCI and AD) made significantly more fixations on the screen before looking for the first time at any stimulus (AD: M=0.97, SD=1.34; MCI:

M=0.87, SD=1.03) compared to controls (M=0.79, SD=0.89; p<0.001). As expected, all groups showed a statistically significantly (p<0.001) higher number of fixations before finding the target stimulus than before finding any of the distractor images, which can be explained by the 3-fold number of distractors present in each trial compared to the (single) target stimulus. Although the interaction 'Group' x 'Stimulus' failed to reach statistical significance, a trend towards this significance was observed, with both AD and MCI patients (AD: M= 3.50, SD=3.37; MCI: M=3.32, SD=2.32) making more fixations before detecting the target stimulus for the first time than cognitively unimpaired subjects (CTRL: M=0.86, SD=0.68; p=0.055). The same oculomotor behavior was observed for the distractors, with both patient groups fixating more times in the screen than controls before finding a distractor for the first time (AD: M= 0.94, SD=1.41; MCI: M=0.85, SD=1.10; CTRL: M=0.78, SD=0.94) (Table 2, Figure 2B).

Fixation count (FC):

Results of the variable FC did not show any main effects of 'Group' (p=0.228) regarding the number of fixations made inside of the ROI when considering all four stimuli as a whole. However, a main effect of 'Stimulus' was observed (p<0.001), with more fixations being made in the distractors than in the target. A 'Group' x 'Stimulus' interaction was observed, both patient groups exhibiting significantly more fixations than controls in the distractor ROIs (AD: M=7.12, SD=9.58; MCI: M=6.46, SD=8.17; CTRL: M=5.46, SD=6.57; p<0.001). The same eye movement pattern was registered in the target ROI, with controls making significantly less saccades (CTRL: M= 5.48, SD=3.09) than MCI and AD. Nonetheless, despite the similar performance, subjects with MCI made slightly more fixations than those with AD (MCI: M=5.90, SD=3.99; AD: M=5.81, SD=4.32) when looking at the target stimulus. Interestingly, CTRL subjects seem to exhibit approximately the same number of fixations, regardless the type of stimulus (Table 2, Figure 2C).

Duration of fixations (DF):

A main effect of 'Group' (p=0.006) and 'Stimulus' (p=0.001) was found for the variable DF (duration of fixations). Controls spent less time fixating on a ROI than patients with MCI and AD, who spent the longest time looking at a specific ROI (CTRL: M= 1.27, SD= 1.85; MCI: M=1.46, SD=2.32; AD: M=1.70, SD=3.01). Subjects (irrespective of group allocation) spent significantly less time fixating on target stimuli than on distractors present in each trial, as seen by the 'Stimulus' effect. 'Group' x 'Stimulus' interaction was statistically significant (p= 0.013). Specifically, both MCI and AD patients spent significantly less time fixating on target stimuli than control subjects (MCI: M=0.70, SD=0.41; AD: M=0.71, SD=0.45; CTRL: M= 0.66, SD= 0.38). Time spent looking at distractor stimuli was significantly different between each group (AD: M=1.99, SD=3.35; MCI: M=1.68, SD=2.58; CTRL: M=1.45, SD=2.06) (Table 2, Figure 2D).

Machine learning classification models:

After filtering all the data and removing the redundancy, 94 non-redundant variables were selected. Feature selection resulted in divergent number of variables. Sequential Feature Selector (SFS) analysis resulted in two variables: variable (1), first fixation duration (FFD) and variable (2), number of fixations before fixating on a target ROI (FBT). The Genetic Algorithm (GA) resulted in 11 variables and Las Vegas Weight (LVW) in 13 variables. Using all 4 datasets (all, SFS, GA and LVW), five algorithms were tested in machine learning. In the present study, we only considered results from models with AUC$\geq$0.75 and accuracy $\geq$0.70 (Table 3). All models were similar to each other; however the model with the best combination of parameters (accuracy, precision and recall) is LVW Random Forest (RF), with 13 features. However, in order to generalize the model, SFS Support Vector Machine (SVM) with radial basis function (RBF) fitted better the purpose of the analysis. The performance of this model was AUC=0.79 with accuracy of 0.72, precision of 0.72 and recall of 0.69 (Figure 3).

Calculations were performed with 10 k-fold, with 90% samples for training and 10% samples for validation.

TABLE 3

FIGURE 3

The distribution of sample values in the variable “number of fixations before” (variable 2, FB) showed to be higher in AD than in controls, while “first fixation duration” (variable 3, FFD) was low for both groups (Figure 4). Only the variable “number of fixations before” (FB) indicated statistically significant higher values in AD as compared to controls (p=0.00022). This variable showed a mean value of 3.21 (SD=0.89) in the control group and 4.45 (SD=1.60) in the AD group, while mean FFD was 0.039 (SD=0.06) in controls and 0.042 (SD=0.07) in AD. When this model was used to re-classify subjects in the MCI group (n=51) as AD or controls, according to the presence of a patterns similar to these other two groups (respectively), this analysis resulted in 25 former MCI subjects re-classified as AD, and 26 former MCI subjects re-classified as controls. In order to verify the potential of this classification, we compared the “number of fixations before” (FB) between groups; we found highly significant statistical differences between AD and controls, the former displaying higher values in this variable as compared to controls (p=1e-9). The mean value for this variable was 3.05 (SD=0.43) for patients with MCI re-classified as AD (MCI-AD subjects) and 4.68(SD=0.79) for those re-classified as controls (MCI-CTRL subjects) (Figure 5).

FIGURE 5

Discussion

The aim of the present study was to investigate the association between eye movement metrics and visual search impairment in a sample of older adults with normal cognitive function and elderly patients with MCI and AD. Using a conventional statistical approach in the analysis of a set of four eyetracking parameters, our results suggest that MCI and AD patients have a similar pattern of visual search impairment, as reflected by an abnormal eye movement behavior. These findings were corroborated by a more sophisticated, large-scale, analytical approach using machine learning methods to address nine eyetracking variables. Therefore, by addressing visual search behavior with the aid of eyetracking technology, we could accurately discriminate patients with mild dementia due to AD from healthy controls, and to further identify, within the MCI group, a subset of patients with an oculomotor behavior similar to the one found in AD.

In general, cognitively unimpaired subjects tended to have a more efficient visual search pattern by looking a similar number of times at both target and distractor stimuli. However, they seem to spend more time in each fixation when analyzing the distractors than when analyzing the target stimulus. Conversely, patients with cognitive impairment (MCI or AD) showed a less efficient visual search behavior. Both MCI and AD fixated more often on the screen when searching for target stimuli and spent more time doing this than controls. Specifically, both patient groups fixated the on distractors more often and for a longer period of time than on the target stimulus. Eye movement control was also not random during the initial screening of the image for all groups. Healthy controls tended to be faster to make the first fixation on the screen, requiring less time to detect distractors than to detect target stimuli. Also, they needed to fixate a smaller number of times on the screen in order to detect any stimulus than cognitively impaired patients in both groups (i.e., MCI and AD). Altogether, these findings seem to be in agreement with the literature confirming that an impairment in

top-down visual processing may be found early in the course of AD.[13,23,36,47] The decline observed in AD patients in visual search has been associated with decreased gray matter in bilateral parietal lobes, precuneus, occipital, temporal, and frontal lobes.[48]

Impairments in time-dependent variables, such as latencies, fixation duration or reaction times, proved to be specific to AD and not just a global slowing of information processing speed due to cognitive ageing.[4,24,49] A decline in visuo-spatial attention resources is responsible for reduced search efficiency and for a potential neglect of important details when searching for a target. Eye movement paradigms are an objective and accurate behavioral response of these deficits in AD.[5,15,50] AD patients reveal slower reaction times due to an inability to move their eyes with the necessary precision and time-efficiency required to perform a thorough scanning of the visual field. Globally, these patients reveal an increase in the number and duration of eye fixations during visual search, narrowing the locus of their attention, which results in deficits on planning their future fixations. This pattern largely contributes to the lengthening of target detection time.[15,24,51] Our findings seem to reflect this oculomotor pattern, with both MC and AD patients not only making more fixations with longer durations, but also making more fixations before finding the target for the first time. This may be explained by slower saccade initiation times due to a decline in the ability to initiate eye movements. Conversely, individuals from the control group showed a much more efficient visual screening, with less fixations before finding the target and with a reduced fixation duration compared to both patient (i.e., cognitively impaired) groups. We can assume that healthy controls were able to limit their random fixations in order to optimize the search for the target ROIs or to optimize their search in the most relevant regions before making a final decision.

In the present study, all three groups distinctively scrutinized targets and distractors. We were able to observe that both patients groups detected distractors faster than target stimuli, spending more time in the corresponding distractor ROIs. This finding was demonstrated by the fact that

MCI and AD subjects, as compared to controls, made an increased number of fixations around the distractors, along with a decreased number of fixations, before looking at any distractor for the first time. In parallel, all three groups revealed increased fixation duration around distractors than in target stimuli, with AD patients spending more time looking at them than the other groups, along with MCI subjects spending more time than controls. Therefore, the present set of data suggests that the effectiveness of visual search, regarding the ability to discard incorrect options in a setting with multiple choices, is inversely related to the degree of cognitive impairment. In the present experiment, subjects were expected to find a target stimulus among a larger number of distractors in the visual scene, invariably in the ratio of three distractor items to one single target item, which represents the effective recall of the stimulus presented in the familiarization phase. These results corroborate the literature demonstrating that the visual search in AD patients were significantly slowed by an increased number of fixations on distractors, which causes a negative impact on visual search efficiency.[52,53] Spatial attention is necessary for accurate perception of complex objects, so impairments in attention could impede stimulus processing. Also, one must acknowledge the fact that similarity between distractors and the target greatly affects AD performance in tasks that depend on memory encoding and subsequent recall. Some authors have advocate that visual search requires high-order processing of multiple features of the target (e.g. shape, color, pattern) (REF.), and these features must be conjoined so that the target can be correctly discriminated from distractors that might share some of these features. Moreover, the higher the number of distractors present in a visual scene, the longest the time required to find the target.[54,55] Feature-conjunction search is known to be mediated by occipitoparietal cortical areas, which are known to be affected by AD pathology.[56] In a study with feature-conjunction tasks conducted by Cormack and colleagues,[52] AD patients made more errors than control subjects in detecting a target when the number of distractors were higher. In the present study,

although subjects with MCI spent significantly less time in the distractor ROI (probably due to a less impaired processing of visual information), they exhibited a number of fixations in the distractor ROI similar to the AD group. This finding suggests that impairments in feature-conjunction processing might be already present at incipient stages of the disease process, such as the MCI condition.

Previous studies have proved that impairments in attentional shifting, combined with a marked slowing in information processing speed, prevents AD patients from effectively searching for a target.[35,57] Interestingly, MCI patients had an altered visual search pattern similar to the one observed in AD. More fixations were made on distractor stimuli than on target stimuli, around which both patient groups (i.e., MCI and AD) made approximately the same number of fixations. In terms of time-dependent measures, MCI subjects had a distinct eye movement pattern, placing themselves between the other two groups in terms of performance. While they presented the same mean values for 'fixation duration' as the other two groups when looking at a given target, they spent less time than AD subjects but more time than controls fixating on distractor stimuli. The same pattern can be observed specifically in the 'time to first fixation' variable, where MCI occupied an intermediary position between controls and AD. One possible explanation for these findings is that an early impairment in attentional shifting can already be found in MCI condition. This precludes the effective scanning a visual scene and the ability to quickly switch between stimuli, as also occurs in AD. Similarly, a failure in the disengagement of attention from distracting stimuli that display a close resemblance to the target stimulus can lead to failures in planning for a strategic visual search. This finding was corroborated by other studies,[36,58] where the task-related selection of the target (top-down control) was already shown to be impaired already in the MCI stage, and declined further in AD. The observed decline in visual search efficiency might result from an early cortical disconnection in the frontoparietal

attention network in patients with MCI, followed by an additional loss of nerve cells in corresponding association areas later on in MCI-AD continuum.[58]

In the current study, we analyzed eye movement variables using a machine learning classification model to: (1) distinguish AD participants from healthy controls and (2) to re-classify MCI subjects into CTRL or AD according to their oculomotor profiles. In particular, Support Vector Machine (SVM) algorithm with radial basis function (RBF) yielded the best classification performance, distinguishing groups with an AUC of 0.79, with a precision of 72% and recall of 69%. In addition, the metric of the variable 'Number of fixations before the first fixation in the target ROI' exhibited the best discrimination between AD and healthy controls. The same model was able to identify subjects with MCI who had an oculomotor performance similar to healthy elders or similar to AD patients in a statistically significant manner (p=1e-9). The literature is still very scarce on the use of machine learning methods applied to eyetracking parameters to ascertain variant conditions related to cognitive decline in the elderly. Nonetheless, previous studies seem to corroborate our results, indicating that these methods can aid the detection of cognitive decline based on the analysis of eye tracking data. In a study with a novelty preference task that measures visual memory impairments, an SVM classification algorithm was able to distinguish MCI patients from healthy controls, using eye movement features.[38] Similarly, a hidden Markov model (HMM) distinguished with good accuracy a sample of young-onset AD patients from healthy controls, by means of the analysis of gaze movements in a smooth pursuit task.[28] Other authors reported the use of deep-learning neural networks in identifying AD patients using a reading task with a good accuracy[59]. The large amount of data that can be extracted from an eye movement experiment raises an important issue in terms of data analyses, given that data extraction and processing can be quite time-consuming. In this sense, computer science has drastically changed how eye movement data are analyzed. Nowadays, this is almost exclusively done by using detection algorithm in

the raw gaze data. Thus, the combination of machine learning techniques and eyetracking technology opens new possibilities in research and/or clinical settings. Considering elderly people and clinical populations, it allows overcoming motor and physical interference in cognitive assessment and it contributes to a more accurate and reliable clinical diagnosis. In combination with other biomarkers, this might help not only to better identify individuals at risk of conversion in a pre-symptomatic stage of AD pathology, but also it allows to closely monitor disease progression over time.

We must acknowledge that the present study may have some potential methodological limitations. First, with respect to sample size, although larger than most studies conducted so far in this field the present sample was relatively small for machine learning classification analyses. Therefore, a larger sample with more eye movement features might have contributed to a better performance of the machine learning algorithms we tested. Second, regarding the characteristics of the patient group: the MCI sample comprised different sub-types (amnestic, non-amnestic and multiple-domain) of MCI, which may not be the best approach to address eye movement profiles in non-demented elders. That is to say, more heterogeneous test groups might be more suitable to ascertain subtle changes in visual search related either with amnestic and non-amnestic impairments. Nonetheless, previous studies[60] exploring visual search impairments in MCI samples have shown altered visual search patterns associated with amnestic MCI patients are not the result of normal ageing, which suggests that visual search impairments may be an underappreciated area of impairment in pre-clinical stages of AD. Third, in the present analysis, oculomotor metrics were not correlated with neuropsychological or other cognitive measures (data not shown), preventing us to draw further conclusions about the underlying cognitive deficits that might affect visual search abilities in MCI and AD. Future studies should include specific neuropsychological measures of visuospatial abilities but also other executive function measures of cognitive flexibility, inhibitory control, visual attention

and working memory that are known to affect eye movement behaviour in these clinical populations.[33,34,61]

In summary, we have shown that eye movement metrics reveal top-down control during visual search and that they can improve the definition of early cognitive decline in pre-symptomatic stages of AD. Our set of data further suggests that the combination of machine learning methods with eye tracking metrics can be a powerful tool not only in the identification of individuals with high-risk of developing neurodegenerative conditions such as AD, but also in tracking disease progression over time.

Conflict of interest

None.

Acknowledgements

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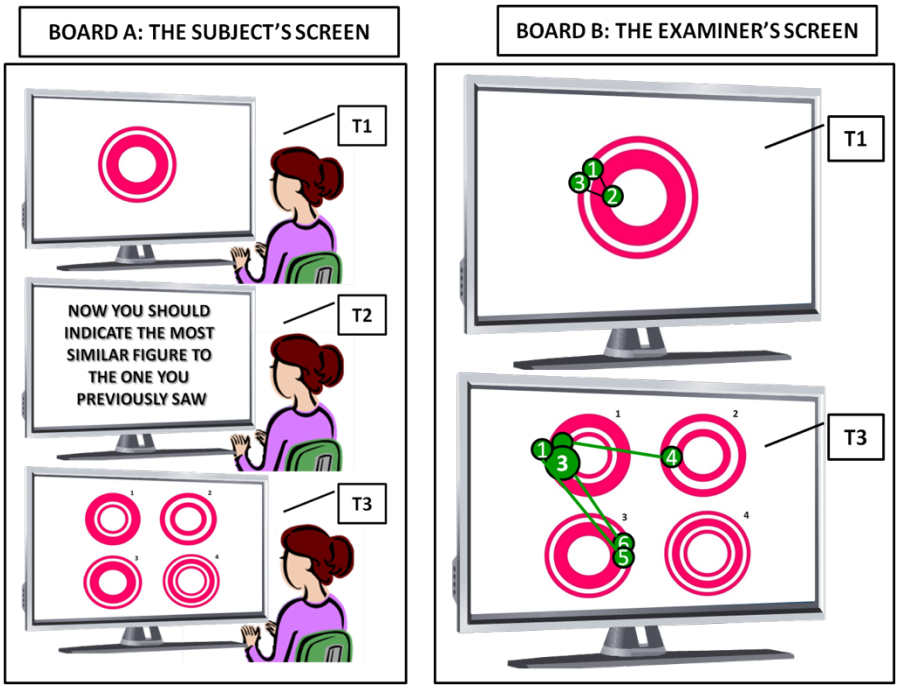

Figure 1. Visual search task.T1, T2, and T3 represent three successive moments during the same task. **Board A.** T1: stimulus presentation; T2: instructions; T3: recognition task. **Board B.** T1 and T3: representations of the eye movement behavior during test phase.

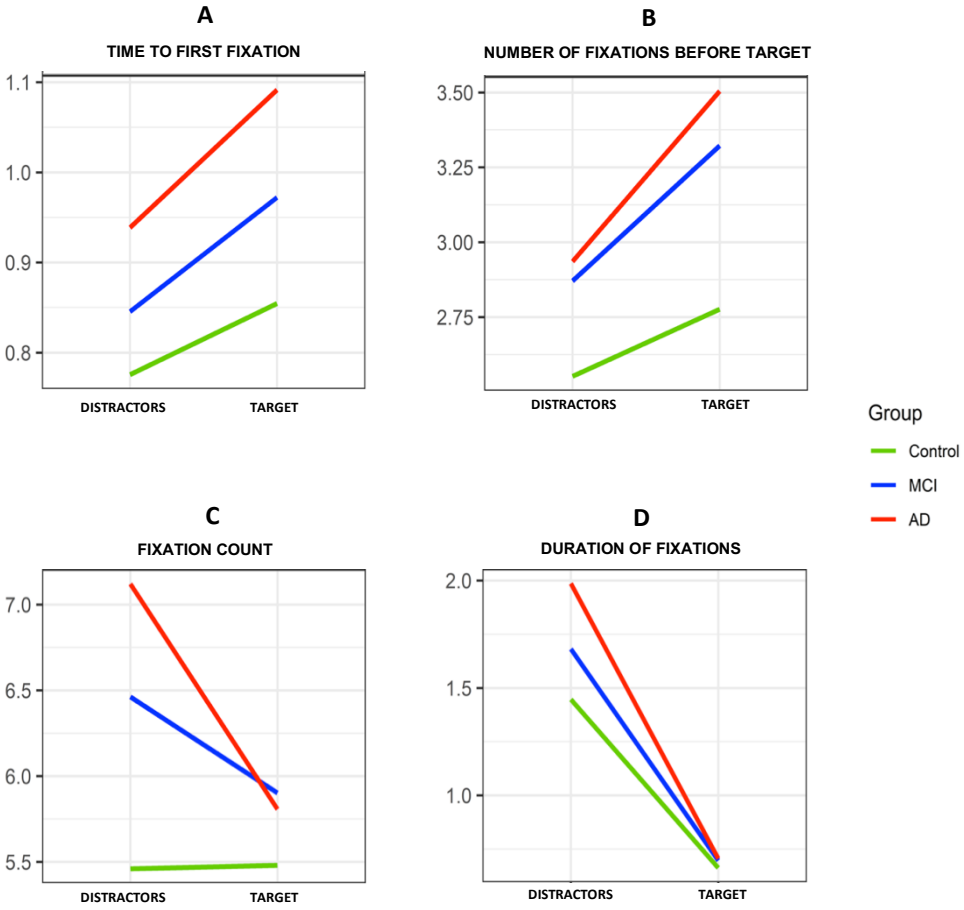

Figure 2. Eye movement metrics in healthy controls, MCI and AD subjects: (A) Time to first fixation (TFF); (B) Number of fixations before target (FB); (C) Fixation count (FC); (D) Duration of fixations (DF).

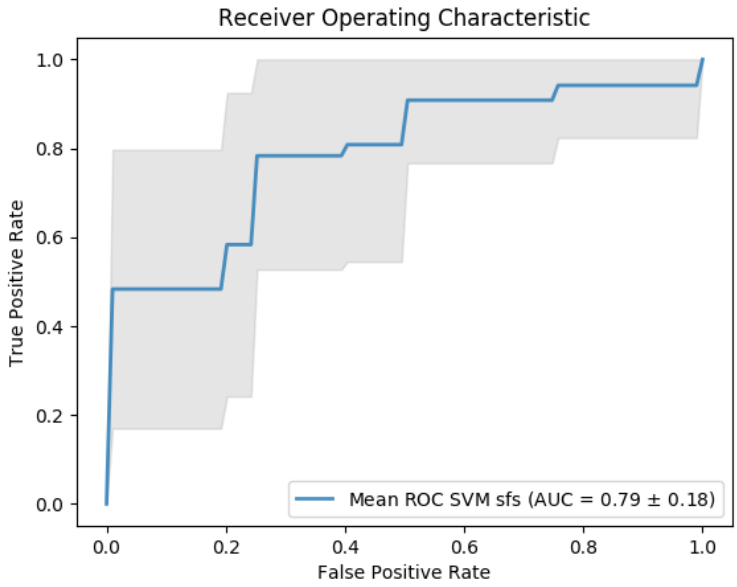

Figure 3: Receiver Operating Characteristic (ROC). Curve using SFS features in SVM RBF model. The AUC is calculated and showed in the bottom. Gray area represents cover for all test performed and blue line represents the mean for all tests performed, which was 10 k-fold.

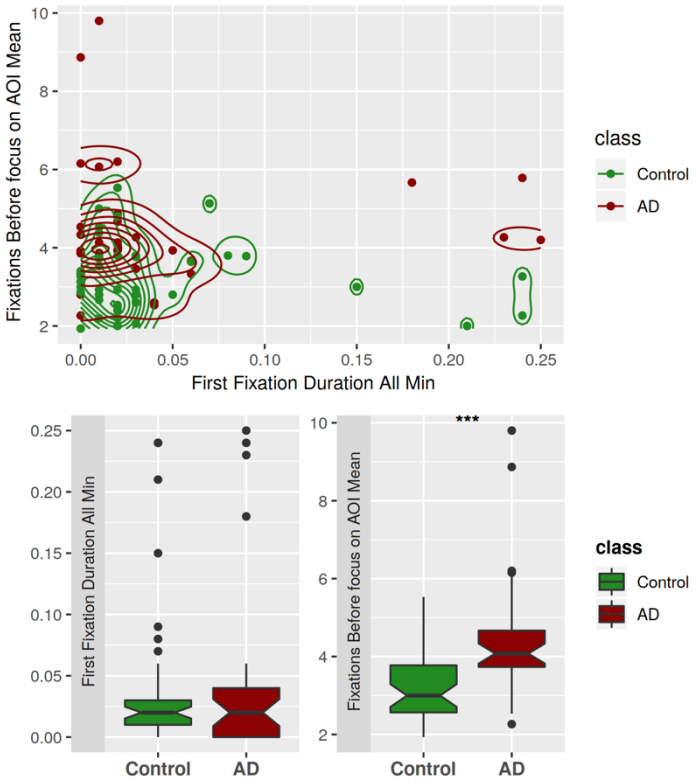

Figure 4: Distribution of SFS variables values into AD and control groups. Upper panel shows a genometric density plot, the areas inside the graphic with geometric figures shows higher amount of samples; x axis represents 'First fixation duration' and y axis represent 'Fixations before focus in AOI', Lower panel shows the boxplot among groups, red represent AD subjects and green represent control subjects; asterisks indicate significance level of p-value (* < 0.05, ** < 0.01, *** < 0.001).

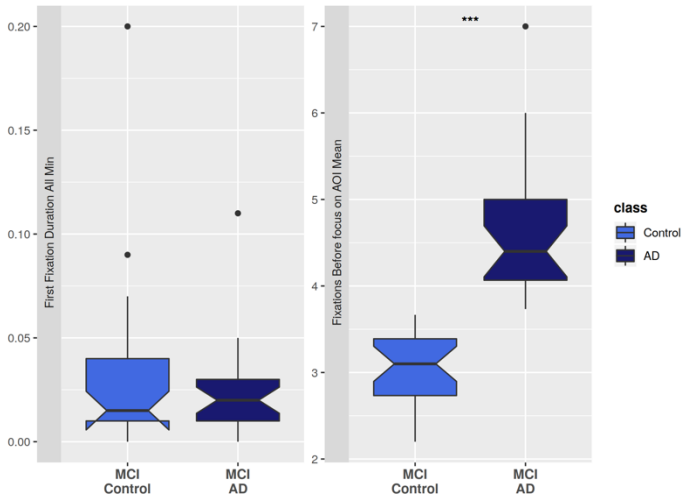

Figure 5: Distribution of variables in MCI subjects classified as AD or Control. Dark blue represents MCI-AD and royal blue MCI-Control; asterisks indicate significance level of p-value (* < 0.05, ** < 0.01, *** < 0.001).

Table 1. Subjects demographics (mean / standard deviation)

	CTRL	MCI	AD	F	K	df	*p**
N	43	52	43***	na		na	na
Age	67.98 (7.15)	68.33 (7.75)	72.97 (6.26)		13.37	2	0.001
Education	15.28 (2.52)	13.06 (3.84)	10.42 (5.23)		22.94	2	<0.001
Gender – F (M)	33 (10)	41 (11)	20 (13)	3.81		2	0.149
RAVLT total	50.45 (6.35)	42.55 (7.85)	26.35 (4.78)	82.61		2.11	<0.001
RAVLT recall	11.00 (2.08)	8.28 (2.87)	2.73 (2.31)	78.61		2.11	<0.001
RAVLT recognition	5.03 (6.60)	2.55 (5.00)	0.64 (2.87)		7.27	2	0.026
TMT-A	39.74 (13.31)	63.31 (28.15)	90.91 (54.32)		32.62	2	<0.001
TMT-B	84.03 (26.87)	142.80 (83.57)	249.11 (152.00)		30.70	2	<0.001
FDS	8.73 (2.17)	7.52 (2.47)	6.55 (1.53)		14.80	2	<0.001
BDS	6.43 (1.58)	4.96 (1.74)	3.91 (1.31)		34.75	2	<0.001
Fluency	43.59 (9.16)	36.78 (11.51)	24.55 (12.78)	21.03		2.11	<0.001
Rey copy	34.36 (2.01)	33.02 (3.28)	25.48 (9.64)		27.42	2	<0.001
Rey recall	16.91 (4.96)	15.42 (6.07)	7.55 (5.73)	20.08		2.10	<0.001

If the data did not comply with the ANOVA parameters (heterogeneity and normality), Kruskal–Wallis K-tests was used as a nonparametric statistical test (K column).

Table 2. Eye movement metrics, group comparisons

METRICS	STIMULUS	GROUP (M/SD)					
		CTRL	MCI	AD	Group	Stimulus	Interaction
Time to First Fixation	Target	0.86 (0.68)	0.97 (0.72)	1.09 (1.06)	<0.001[1]	<0.001[1]	0.600[1]
	Distractors	0.78 (0.94)	0.85 (1.10)	0.94 (1.41)			
	Total	0.79 (0.89)	0.87 (1.03)	0.97 (1.34)			
Fixations Before	Target	2.78 (2.12)	3.32 (2.32)	3.50 (3.37)	<0.001[2]	<0.001[2]	0.055[2]
	Distractors	2.55 (3.12)	2.87 (3.67)	2.94 (4.12)			
	Total	2.60 (2.93)	2.97 (3.43)	3.06 (3.97)			
Fixation Count	Target	5.48 (3.09)	5.90 (3.99)	5.81 (4.32)	0.228[2]	<0.001[2]	<0.001[2]
	Distractors	5.46 (6.57)	6.46 (8.17)	7.12 (9.58)			
	Total	5.46 (6.00)	6.34 (7.48)	6.84 (8.75)			
Duration of Fixations	Target	0.66 (0.38)	0.70 (0.41)	0.71 (0.45)	0.006[1]	<0.001[1]	0.013[1]
	Distractors	1.45 (2.06)	1.68 (2.58)	1.99 (3.35)			
	Total	1.27 (1.85)	1.46 (2.32)	1.70 (3.01)			

1. Linear mixed effect models, 2. Generalized linear mixed model for Poisson family, 3. Generalized linear mixed model for binomial family.

Table 3: Summary of classification model performance.

Data	AUC	Accuracy	Error	Precision	Recall	F1-score
Feature model SFS (n =2)						
SVM RBF	0.79 ± 0.18	0.72 ± 0.14	0.28 ± 0.14	0.72 ± 0.22	0.69 ± 0.16	0.69 ± 0.15
Feature model GA (n = 11)						
RF	0.79 ± 0.19	0.76 ± 0.1	0.24 ± 0.1	0.78 ± 0.17	0.67 ± 0.23	0.69 ± 0.16
Feature model LVW (n = 13)						
RF	0.77 ± 0.19	0.8 ± 0.14	0.2 ± 0.14	0.83 ± 0.19	0.7 ± 0.26	0.74 ± 0.2
NN	0.8 ± 0.12	0.79 ± 0.11	0.21 ± 0.11	0.83 ± 0.19	0.67 ± 0.17	0.73 ± 0.15
Feature model ALL (n =94)						
SVM Linear	0.8 ± 0.13	0.74 ± 0.11	0.26 ± 0.11	0.76 ± 0.18	0.62 ± 0.22	0.66 ± 0.17
RF	0.8 ± 0.18	0.78 ± 0.12	0.22 ± 0.12	0.83 ± 0.2	0.67 ± 0.23	0.71 ± 0.17